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STUDIES ON ADRENALINE AND NORADRENALINE IN HUMAN PLASMA

BY

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LUND 1960

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HÅKAN OHLSSONS BOKTRYCKERI

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Introduction

Much of our knowledge in the field of catechol amines has been attained by biological methods of assay. The concentrations of adrenaline and noradrenaline in the suprarenal venous blood of experimental animals are frequently so high that direct estimation can be achieved by using bioassay procedures for these physiologically active substances.

The determination of adrenaline and noradrenaline in the peripheral blood is much more difficult, and attempts to isolate catechol amines from peripheral blood or to prepare these amines in such quantities that a wholly convincing set of biological or chemical tests could be undertaken meet great difficulties, the amounts present being so small.

The importance of a more sensitive and specific method for determination of small amounts of catechol amines is obvious, however. Of all methods currently available for estimating the concentrations of catechol amines in the blood, only fluorometric methods appear to be sensitive enough to allow the simultaneous chemical determination of the small amounts of these amines normally present in peripheral blood. A disadvantage of any chemical method, however, is the fact that no knowledge of the biological activity of the substances being measured is obtained.

In recent years, the advances made in the methods of identification and quantitative estimation of catechol amines have led to general interest in the physiology of the sympathetic nervous system and in the suprarenal medulla in health and disease. Additional information regarding the temporal relationship between physiological sequences or various disease processes and the amounts of catechol amines released into the circulation would improve the understanding of these neurohormones, as the changes may be sudden and transient and detected only by measuring the plasma concentrations.

In the present work a spectrophotofluorometric method for determination of the minute amounts of catechol amines in human plasma is presented. The method is applied to problems both of physiology and of clinical medicine, in certain conditions where a deeper understanding of the role of the sympathetic nervous system is desirable.

Chapter I

Review of Literature

Historical Remarks

Secretion of a specific chemical substance from the suprarenal medulla was traced by VULPIAN (1856), who described the characteristic colour reactions in the medulla and in the suprarenal venous blood on oxidation. In the light of what we know now Vulpian probably demonstrated, for the first time, the storage and release of catechol amines.

The physiological activity of extracts of the suprarenal medulla was described by OLIVER and SCHÄFER (1895), SZYMONOWICZ (1895), and CYBULSKI (1895). The latter demonstrated the presence of the active substance in suprarenal venous blood under conditions of stress and concluded that it was secreted by the gland. Cybulski also showed that extracts of the suprarenal medulla, when injected intravenously, increased the pressor activity of the urine.

Adrenaline, identified as one of the active principles in the suprarenal medulla by TAKAMINE (1901) and synthesized together with its primary amino-homologue, noradrenaline, by STOLZ (1904), was long considered to be not only the sole specific hormone of the suprarenal medulla but also the neurohumoral transmitter of sympathetic nervous impulses, liberated at the nerve endings (the pertinent literature has been reviewed by TAINTER and LUDUENA, 1950, and EULER, 1956). However, as early as in 1910 BARGER and DALE questioned the identification of adrenaline in the sympathetic neurohormone. The discrepancies noted between the effects of sympathetic nerve stimulation and the action of adrenaline led to the assumption of two different active substances, sympathin I and E, owing to their inhibitory and excitatory actions. They were thought to be formed by the interaction of a primary chemical mediator, adrenaline or an adrenaline-like substance, with a hypothetical receptive constituent of the reacting cells (CANNON and ROSENBLUETH, 1933).

The comparative studies on the effects of sympathomimetic amines and on stimulation of adrenergic nerves, however, led to the idea that noradrenaline might be responsible for the excitatory reactions and adrenaline for the inhibitory ones (BACQ, 1934, MELVILLE, 1937, STEHLE

and ELLSWORTH, 1937, and GREER, PINKSTON, BAXTER and BRANNON, 1938).

EULER (1946) showed with improved biological methods that the active principle of sympathetic ganglia and nerves, and organs innervated by such nerves, closely resembles noradrenaline. The identification of the specific active substance with laevo-noradrenaline was later achieved by various biological and chemical methods (EULER, 1948). The results of studies on the release of the active substances by adrenergic nerve stimulation *in vivo* provided evidence that direct stimulation of the adrenergic nerves to the organ is followed mainly by noradrenaline activity in the effluent blood and that only very small amounts, if any, of adrenaline are present in addition to noradrenaline (PEART, 1949, MANN and WEST, 1950, 1951).

As a result of these findings the idea that noradrenaline is of prime importance in the chemical mediation of adrenergic nerve impulses became generally accepted.

The demonstration of noradrenaline as an active principle in the normal suprarenal medulla originated from a suggestion by HOLTZ, CREDNER and KRONEBERG (1947). BERGSTRÖM, EULER and HAMBERG (1949) isolated noradrenaline from extracts of suprarenal glands, and showed that it conformed with the synthetic laevo-compound, previously prepared by TULLAR (1949). This proved its biological origin. The presence of noradrenaline (as well as adrenaline) in the medulla of most vertebrates has since been repeatedly confirmed, and so has its secretion by the gland (see EULER, 1956). The presence of both adrenaline and noradrenaline in the outflow from the suprarenal medulla has been taken to indicate a dual function of the medullary secretion.

A third amine, dopamine, the primary product of the action of l-dopa-decarboxylase (HOLTZ, HEISE and LÜDTKE, 1938), was recognized by HOLTZ, CREDNER and KOEPP (1942) as a naturally occurring substance in human urine. Using different biological methods, HOLTZ, CREDNER and KRONEBERG (1947) were the first to assume that the active amines in normal urine of man consist of a mixture of adrenaline, noradrenaline and dopamine. This was later confirmed by chromatographic analysis by EULER and HELLNER (1951). Dopamine has since been identified in suprarenals (GOODALL, 1950, SHEPHERD and WEST, 1953) and in other tissues of ruminants (GOODALL, 1950, EULER and LISHAJKO, 1957, SCHÜMANN, 1958), in ruminant sympathetic nerve fibers (SCHÜMANN, 1956, 1958) and in brain of different species including man (WEIL-MALHERBE and BONE, 1957. a, CARLSSON, LINDQVIST, MAGNUSSON and WALDECK, 1958, BERTLER and ROSENGREN, 1959. a).

As early as 1939 BLASCHKO recognized that noradrenaline must occur as an immediate precursor in the biosynthesis of adrenaline. More recent experiments have now established the reactions involved in the biosynthesis of adrenaline from noradrenaline. Thus noradrenaline plays a dual role, not only as a substance with an independent biological action, but also as an intermediate, *i.e.*, in adrenaline formation. As for dopamine, it is not yet clear whether it has a similarly dual role. Its occurrence as a precursor of noradrenaline and adrenaline is established. The fact that dopamine and noradrenaline show markedly different distributions in lungs of ruminants (EULER and LISHAJKO, 1957) and in the brain of several mammalian species (BERTLER and ROSENGREN, 1959. b), makes it necessary to consider the possibility that the third catechol amine is not merely a precursor.

General Distribution of Catechol Amines in the Body

Catechol amines adrenaline, noradrenaline and dopamine are found in the chromaffin tissues, in postganglionic sympathetic neurones of the adrenergic type and in certain parts of the central nervous system, the proportion of each amine present varying from species to species.

Chromaffin Tissues

The specialized chromaffin tissue which is present mainly in the suprarenal medulla and is found at a late stage of intra-uterine life in the organs of Zuckerkandl (generally recognized as belonging to the chromaffin system) accumulates catechol amines. The relative proportions of adrenaline and noradrenaline in suprarenal glands appear to be fairly constant and characteristic of each species (EULER, 1956). This is of interest in view of reports on the presence of separate adrenaline- and noradrenaline-storing cells in suprarenal glands (HILLARP and HÖKFELT, 1953, 1954, ERÄNKÖ, 1955). Human suprarenal glands removed by surgery have been found to contain 0.5 mg of adrenaline and 0.1 mg of noradrenaline per gram tissue, the average percentage adrenaline amounting to 84 per cent of the total catechol amines (EULER, FRANKSSON and HELLSTRÖM, 1953). Noradrenaline is the predominant suprarenal catechol amine in fetal and neonatal life (HÖKFELT, 1951, SHEPHERD and WEST, 1951), the proportion of adrenaline showing a steady increase with maturation of the gland (WEST, SHEPHERD and HUNTER, 1951).

In the ruminant suprarenal medulla dopamine provides only a small

percentage of the total catechol amine content (DENGLER, 1957). Attempts to demonstrate its presence in suprarenal glands of other species have not been successful.

Adrenaline and noradrenaline are largely kept within intracellular granules (HILLARP, LAGERSTEDT and NILSON 1953, BLASCHKO and WELCH, 1953) other than mitochondria (BLASCHKO, HAGEN and HAGEN, 1957, EADE, 1956). The distribution of dopamine in the bovine suprarenal medulla appears to be very similar to that of adrenaline and noradrenaline (EADE, 1958). There seems to be good evidence that the largest fraction of catechol amines is stored in granules in a bound state in combination with an equivalent amount of adenosinephosphates, mainly ATP (adenosinetriphosphate) (FALCK, HILLARP and HÖGBERG, 1956, CARLSSON and HILLARP, 1956, BLASCHKO, BORN, D'IORIO and EADE, 1956, BLASCHKO, HAGEN and HAGEN, 1957, HILLARP, 1958, 1959). The granules probably serve only as structures for the storage of amines (and possibly ATP) in a readily available form.

The granules can be made to release their catechol amines by stimulation *via* the splanchnic nerves (CARLSSON and HILLARP, 1956), by changes in the tonicity and in the pH of suspending solution, and with certain detergents or histamine-liberators (HILLARP and NILSON, 1954, EADE, 1957). A release of the catechol amines is accompanied by a concomitant release of an equivalent amount of adenosinephosphates. The way in which the released catechol amines are transported out of the cell is unknown.

The organs of Zuckerkandl contain only noradrenaline at birth and the amount exceeds that in the suprarenals (WEST, SHEPHERD, HUNTER and MACGREGOR, 1953). The relative proportion of adrenaline rises during the first two years of life. The histological and histochemical observations indicate that the organs reach cytological maturity after 5 months of intrauterine life and persist until birth, after which they undergo gradual regression (WEST, SHEPHERD, HUNTER and MACGREGOR, 1953). At the age of 3 years there is usually only little activity left. The organs of Zuckerkandl are regarded as the main source of catechol amines in the foetus and in the newly-born (HUNTER, MACGREGOR, SHEPHERD and WEST, 1952). No knowledge is available as regards the storage of catechol amines in these bodies.

Very little is known with regard to the distribution of adrenaline and noradrenaline in scattered chromaffin cells in various tissues and organs. It has been concluded from indirect evidence that these cells contain adrenaline (EULER and PURKHOLD, 1951, GOODALL, 1951). A widespread system of special chromaffin cells, particularly in human skin, has been

demonstrated by ADAMS-RAY and NORDENSTAM (1956, 1957). No knowledge is available concerning the function of this system or the nature of the chemical composition of these cells. Recent findings (BERTLER, FALCK, HILLARP, ROSENGREN and TORP, 1959, FALCK, HILLARP and TORP, 1959) indicate that the dopamine found in certain tissues and organs of ruminants is stored in special chromaffin cells and that there is a close correlation between the distribution of dopamine and these cells within certain organs.

Adrenergic Nerves

Adrenergic nerves and sympathetic ganglia con'ain only a few (up to 10—15) micrograms of catechol amines per gram tissue, predominantly noradrenaline, and only questionable amounts of adrenaline (see EULER, 1956, and references). Within different kinds of peripheral nerves the content of noradrenaline is dependent on the relative proportion of adrenergic nerve fibers (EULER, 1956). Normally the amount of noradrenaline in any one nerve is characteristic and relatively constant, suggesting that the axones possess a well-defined storing capacity. Noradrenaline is located in the granules isolated from adrenergic neurones (EULER, 1958. a) together with ATP (SCHÜMANN, 1958), the mechanism of the binding of the adrenergic mediator thus closely resembling that of the suprarenal medullary hormones. In ruminants about half of the total catechol amines is noradrenaline and the remainder is dopamine (SCHÜMANN, 1956, EULER and LISHAJKO, 1957). It should perhaps be mentioned that the special chromaffin cells probably storing dopamine lie scattered throughout the adrenergic nerves (splenic nerves) of ruminants (BERTLER, FALCK, HILLARP, ROSENGREN and TORP, 1959).

Peripheral Tumours of the Neurone Series

Tumours taking their origin in the chromaffin cells of the suprarenal medulla or aberrant suprarenal tissue, or in sites where their presence is to be expected on developmental grounds, namely, in association with the prevertebral and peripheral ganglia, may arise from the primitive stages of these cells (phaeochromoblasts or, alternatively, sympathoblasts or neuroblasts), or they may be made up of adult cell type (phaeochromocytomas or chromaffinomas or, alternatively, ganglioneuromas) (Figure 1). The tumours as a rule constitute distinct biological and histological entities but may sometimes be of a transitional or a mixed character (RUSSEL and RUBINSTEIN, 1959).

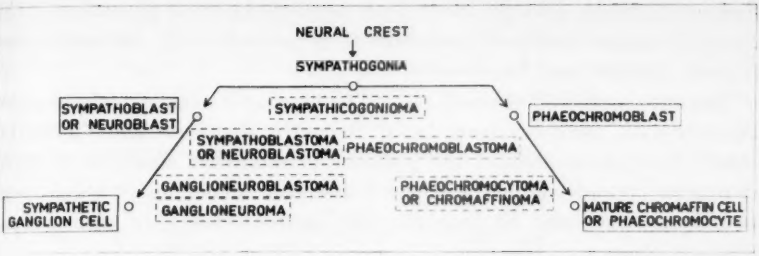


Figure 1. Maturation of sympathogonia takes place along two divergent lines: towards adult sympathetic nerve cells through intermediate sympathoblasts and, in the suprarenal medulla, towards chromaffin cells through the development of phaeochromoblasts. Tumour cells derived from corresponding immature of mature cells are shown in the broken lines.

The capacity of the chromaffinomas for storing catechol amines is high: most of the tumours contain 0.5 to 10 mg of total catechol amines per gram tissue. The majority of tumours accumulate only noradrenaline; yet this amine constitutes only 15—20 per cent of the total catechol amines of the normal suprarenal medulla. While pure noradrenaline tumours and mixed noradrenaline-adrenaline types have been frequently described, no case of a pure adrenaline-producing tumour, however, has so far been encountered. Dopamine in large amounts may be present in tumours (MANGER *et al.*, 1956, MCMILLAN, 1956, WEIL-MALHERBE, 1956). The tumour cell usually secretes catechol amines at a high rate and more or less continously (EULER and STRÖM, 1957, EULER, 1958). The reason for this abnormal secretion behaviour is largely unknown. Recent findings have shown that the amount of ATP in the tumour tissue is about thirty times lower than in the normal suprarenal medulla (HILLARP, LINDQVIST and VENDSALU, 1960), and that at most a small part of the catechol amines could have been stored in the same way as in the normal chromaffin cell, *viz.* in combination with an equivalent amount of adenosinephosphates. The tumour cell thus seems to have a defective storage mechanism.

The measurement of increased output of catechol amines, or their metabolites, in urine provides a reliable basis for the diagnosis of chromaffinoma (see EULER, 1956, and references, ARMSTRONG, MCMILLAN and SHAW, 1957, LA BROSSE, AXELROD and SJOERDSMA, 1958, STUDNITZ, 1960). Free adrenaline and noradrenaline account for 0.4—6.7 per cent, the 3-methoxy analogues for 17—42 per cent, and 3-methoxy-4-

hydroxymandelic acid for 57—78 per cent of the total excretion of the catechol amines and their metabolites in patients with chromaffinoma (CROUT, PISANO and SJOERDSMA, 1960).

Tumours composed of primitive cell forms may contain catechol amines (MASON *et al.*, 1957, GRAHAM, 1959). Recently, STUDNITZ and VENDSALU (1960) have demonstrated the presence of increased amounts of dopa, dopamine, noradrenaline, and the 3-methoxy analogues of these compounds in the urine of patients with neuroblastoma, or with sympathicogonioma.

Tumours originating from adult sympathetic nerve cells have been found to cause increased urinary excretion of noradrenaline and 3-methoxy-4-hydroxymandelic acid (GREENBERG and GARDNER, 1959).

Central Nervous System

In mammalian brain noradrenaline and dopamine are present in about equal quantities. The dopamine content in different species range from 0.19 μg to 0.60 μg per gram fresh tissue and is generally somewhat higher than that of noradrenaline (BERTLER and ROSENGREN, 1959). Adrenaline occurs in small amounts and does not seem to constitute more than a few per cent of the total catechol amine content. The distribution of noradrenaline and dopamine is markedly different. Relatively high concentrations of noradrenaline have been demonstrated in the brain stem, notably the hypothalamus, whereas practically all of the dopamine occurs in the corpus striatum.

The histological localization of these amines is not known. There seems to be evidence that the sympatho-adrenergic nerves do not significantly contribute to the noradrenaline content of the brain (BERTLER and ROSENGREN, 1959. b). Observations by FALCK and HILLARP (1959) suggest that noradrenaline and dopamine in the brain are not stored in typical chromaffin cells.

The observation by WALASZEK and ABOOD (1957), WEIL-MALHERBE and BONE (1957. a), and BERTLER and ROSENGREN (1959. c) seem to indicate that 5-hydroxytryptamine, noradrenaline and dopamine are granule bound.

Biosynthesis

The reactions in the main pathway for biosynthesis of adrenaline seem to proceed through the following steps: phenylalanine — tyrosine — 3,4-dihydroxyphenylalanine (dopa) — 3,4-dihydroxyphenylethylamine (dopamine) — noradrenaline — adrenaline (Figure 2). This sequence of

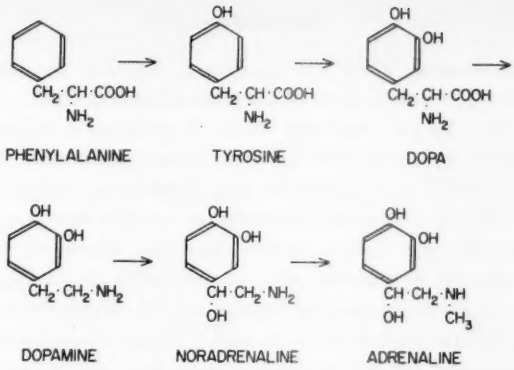


Figure 2. Biosynthetic pathway to adrenaline and noradrenaline.

reactions has been confirmed by experiments with isotopically labelled compounds in intact animals (GURIN and DELLUVA, 1947, UDENFRIEND, COOPER, CLARK and BAER, 1953, UDENFRIEND and WYNGAARDEN, 1956, LEEPER and UDENFRIEND, 1956, MASUOKA, SCHOTT, AKAWIE and CLARK, 1956), on intact suprarenals (ROSENFELD, LEEPER and UDENFRIEND, 1958), on slices or homogenates of suprarenal medulla (DEMIS, BLASCHKO and WELCH, 1955, 1956, HAGEN and WELCH, 1955, HAGEN, 1956, GOODALL and KIRSHNER, 1957. a, PELLERIN and D'IORIO, 1957), and in sympathetic nerves and ganglia (GOODALL and KIRSHNER, 1957. b, 1958). Studies on patients with phaeochromocytoma have led to confirmation of some of these reactions in man (SJOERDSMA, LEEPER, TERRY and UDENFRIEND, 1959). The findings of dopa (WEIL-MALHERBE, 1956), and dopamine (MCMILLAN, 1956, WEIL-MALHERBE, 1956) in tumour tissues provide connecting links which support the idea that these compounds are intermediates in the formation of noradrenaline and adrenaline by chromaffin tissue. Recently, dopa has been found to occur in the urine of human subjects with neuroblastoma, or with sympathicogonioma (STUDNITZ and VENDSALU, 1960).

The adrenergic nerves and sympathetic ganglia synthesize noradrenaline in a systematic sequence from tyrosine and this synthesis apparently terminates with noradrenaline rather than proceeding to the formation of adrenaline (GOODALL and KIRSHNER, 1958).

The exact nature of some of the intermediate chemical reactions in the main pathway of the formation of catechol amines is still little explored, *e.g.* the introduction of the sidechain hydroxyl group.

Metabolism

Until quite recently little was known as regards the nature of the end-products of the metabolism of catechol amines. In 1957, ARMSTRONG, MCMILLAN and SHAW reported that 3-methoxy-4-hydroxymandelic acid was a major metabolic product of noradrenaline in man. Subsequently, AXELROD (1957), and AXELROD and TOMCHICK (1958) described an enzyme, the catechol-O-methyl transferase, widely distributed among a variety of species and tissues, which catalyzes the methylation of the 3-hydroxy position of catechol amines. Normally occurring compounds such as adrenaline, noradrenaline, dopamine, 3,4-dihydroxyphenylalanine (dopa), 3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylacetic acid served as substrates for the enzyme *in vitro*, and studies *in vivo* showed that adrenaline, noradrenaline and dopamine were O-methylated by animal tissues to yield the corresponding 3-methoxy analogues. These were then either conjugated or deaminated and oxidized to the corresponding acids, 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), respectively. 3-Methoxy analogues of adrenaline and noradrenaline were found to be normal constituents in urine and certain tissues of the rat. 3-Methoxynoradrenaline was found to occur in the tumour tissues of human subjects with phaeochromocytomas, in one tumour the 3-methoxy analogue of dopamine was tentatively identified (SJOERDSMA, KING, LEEPER and UDENFRIEND, 1958). More recently, 3-methoxy analogues of dopa and dopamine have been found in the urine of patients with sympathogonioma and neuroblastoma (STUDNITZ and VENDSALU, 1960).

The studies of AXELROD (1957, 1958), and AXELROD and TOMCHICK (1958) afford evidence that O-methylation of catechol amines prior to oxidative deamination is a principal pathway for their metabolism. The importance of monoamine oxidase in the direct oxidation of catechol amines other than dopamine is controversial. Although the work of SCHAYER (1951), and of SCHAYER, SMILEY and KAPLAN (1952) indicate that some of the metabolites of administered ^{14}C-labelled adrenaline are probably formed by oxidative deamination, the slow rate of oxidation of adrenaline and noradrenaline by monoamine oxidase preparations (BLASCHKO, RICHTER and SCHLOSSMAN, 1937) raise doubts as to whether this is an important *in-vivo* mechanism of inactivation. LEEPER, WEISSBACH and UDENFRIEND (1958) have shown that O-methylation of noradrenaline converts it to a substance which is more effectively metabolized by monoamine oxidase. The findings of 3,4-dihydroxymandelic acid in humans (RESNICK, WOLFF, FREEMAN and ELMADJIAN, 1958,

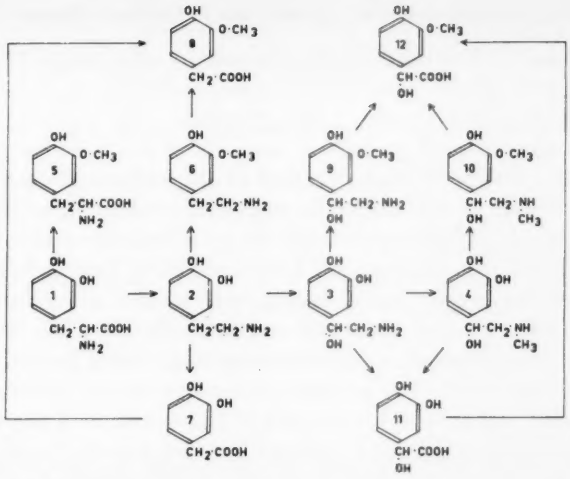


Figure 3. Metabolic pathways involving catechol amines and related substances.

1: 3,4-dihydroxyphenylalanine (dopa), *2*: 3,4-dihydroxyphenylethylamine (dopamine), *3*: noradrenaline, *4*: adrenaline, *5*: 3-methoxy-4-hydroxyphenylalanine, *6*: 3-methoxy-4-hydroxyphenylethylamine, *7*: 3,4-dihydroxyphenylacetic acid, *8*: 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), *9*: 3-methoxynoradrenaline, *10*: 3-methoxyadrenaline, *11*: 3,4-dihydroxymandelic acid, *12*: 3-methoxy-4-hydroxymandelic acid.

Goodall, Rosen and Kirshner, 1958, Kirshner, Klingman and Goodall, 1958, Armstrong and McMillan, 1959, Euler, Floding and Lishajko, 1959) indicate that at least some endogenous adrenaline and noradrenaline undergoes destruction by amine oxidase before methylation occurs. It may well be that the quantitive importance of the methylation *versus* the amine oxidase pathway for the metabolism of catechol amines varies in different tissues and species. The issue is not settled.

Conjugation plays a minor role in the inactivation of physiological amounts of catechol amines (Euler and Luft, 1951, Euler, Luft and Sundin, 1953, Schayer, 1951).

Figure 3 outlines the pathways which now appear to be involved to a major extent in the metabolism of catechol amines.

Catechol Amines and their Metabolites in Normal Human Urine

The amount of free noradrenaline in human urine ranges from about 30 to 65 μg per 24 hours when fluorometrical methods of determination are used (LISHAJKO and EULER, 1959, CARLSSON and VENDSALU, 1959), while free adrenaline is present in amounts of 5—10 to 20 μg, a ratio which is the reverse of that obtained in the suprarenal medulla. The urinary excretion of free dopamine ranges from about 70 to 160 μg and of free dopac (3,4-dihydroxyphenylacetic acid) from about 200 to 380 μg per 24 hours (EULER, FLODING and LISHAJKO, 1959). Besides free catechol amines, varying proportions of conjugated catechol amines are present in normal urine (HOLTZ, CREDNER and KRONEBERG, 1947, EULER and HELLNER, 1951, ELMADJIAN, LAMSON and NERI, 1956, EULER, FLODING and LISHAJKO, 1959). The average percentage of free adrenaline and noradrenaline amounts to 1.4 per cent of the excretion of the metabolic end-product of these amines, 3-methoxy-4-hydroxymandelic acid (average excretion 4.8 mg per 24 hours; STUDNITZ, 1960). 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), to a major part assumed to be formed from dopamine and dopa, is excreted in amounts of 3 to 8 mg in the course of a 24-hour period (SHAW, MCMILLAN and ARMSTRONG, 1957). The daily excretion of free and conjugated 3-methoxy analogues of adrenaline and noradrenaline in normal urine is less than 1 mg per day (PISANO, 1960).

The Development of the Chemical and Physical Methods for Measuring Catechol Amines in Blood

Much of our knowledge in the field of catechol amines has been attained by biological methods of assay. By an appropriate choice of pharmacological methods of estimation and by using parallel tests, the bioassay procedures can be sensitive and specific, and afford valuable information on the qualitative nature of the physiologically active substances in various tissue extracts and urine. The methods do not admit of estimation of very small quantities of adrenaline and noradrenaline present in normal peripheral blood, although estimations can be made during special conditions (HOLZBAUER and VOGT, 1954).

The first attempts to measure catechol amines in human blood by chemical methods were made 25 years ago (WHITEHORN, 1935, SHAW, 1938). Since then numerous procedures utilizing a variety of principles have been reported. Attempts to utilize the colorimetric methods for the quantitative estimation of catechol amines were unsuccessful owing to

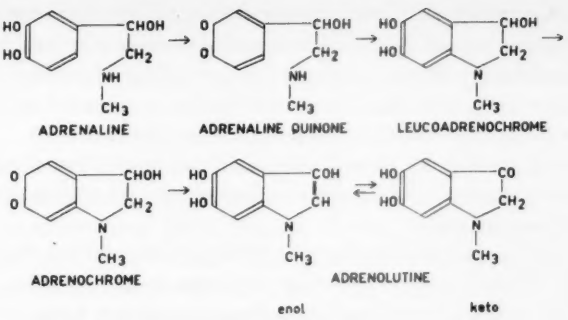


Figure 4. Conversion of adrenaline to adrenolutine.

lack of sensitivity, or specificity. The need for more sensitive, accurate and specific methods for catechol amines brought fluorescent procedures into use in the early 1940's (HUEBER, 1940, KALAJA and SAVOLAINEN, 1941, LEHMANN and MICHAELIS, 1943), based on the observation by LOEW (1918) and PAGET (1930) that adrenaline in alkaline solution gives a transitory yellow-green flourescence. The method, however, gave widely varying results owing to the fluorescent contaminants and to the instability of the fluorescent compound.

Adrenaline, on oxidation, rapidly cyclizes to adrenochrome (RICHTER, 1937, RICHTER and BLASCHKO, 1937). The studies of EHRLÉN (1948), LUND (1949), FISCHER (1949) and HARLEY-MASON (1950) showed that the fluorescent compound formed by addition of alkali to adrenochrome is an isomeric transformation product having the composition of 1-methyl-3,5,6-trihydroxyindole (adrenolutine, Figure 4). Although the isomeric transformation product of noradrenochrome, 3,5,6-trihydroxyindole (noradrenolutine), has not been isolated, there is little doubt that such a product is formed (BU'LOCK and HARLEY-MASON, 1951).

For the estimation of adrenaline in solutions, EHRLÉN (1948) introduced the important procedure of adding ascorbic acid for stabilization of the trihydroxyindoles, which made the method really useful for assay purposes. Using a procedure based on the same principle, LUND (1950) further developed the method and adapted it for estimation of adrenaline and noradrenaline in blood. The catechol amines were isolated by adsorption onto alumina, originally devised by SHAW (1937), and subsequently eluted with acetic acid. The differential analysis for adrenaline and noradrenaline was based upon the pH-controlled differences in

the oxidation rates of these amines, followed by tautomerization to their trihydroxyindoles by alkali. Manganese dioxide was used as oxidant. The low sensitivity of the method did not admit of estimation of the concentrations of adrenaline and noradrenaline in normal blood although estimations could be made during special conditions.

The technique for the quantitative estimation of adrenaline and noradrenaline in plasma has since been improved and the sensitivity and accuracy have increased.

A variant applied by PRICE and PRICE (1957) differs from the trihydroxyindole fluorometric method of LUND in relying upon excitation of solutions of adrenolutine and noradrenolutine with light of two wavelengths rather than upon oxidation at two pH levels for the differential estimation of adrenaline and noradrenaline in a mixture. Potassium ferricyanide was used as an oxidizing agent. Acidifying the reaction products from the strongly alkaline reaction increased the fluorescence of catechol amines. Sensitivity was thereby increased. The average concentration of adrenaline in venous plasma was 0.01 ± 0.07 µg, while that of noradrenaline was 0.34 ± 0.15 µg per litre of plasma.

COHEN and GOLDENBERG (1957) also found that the fluorescent characteristics of adrenolutine and of noradrenolutine were sufficiently different to admit of quantitation of each of these constituents in a mixture if a fluorometric measurement using multiple-filter technique was employed. Oxidation of the amines in the eluate from alumina was achieved with manganese dioxide. The amounts found in normal human venous plasma were 0.06 ± 0.05µg adrenaline and 0.30 ± 0.07 µg noradrenaline per litre plasma.

It is now obvious that only fluorescence affords the required sensitivity for the quantitative determination of catechol amines in plasma. With the development of spectrophotofluorometers it is now possible to attain a higher degree of specificity, and the availability of full activation and fluorescence spectra provides criteria for evaluating specificity and for detecting the presence of interfering substances.

The activation spectra of the trihydroxyindoles derived from adrenaline and noradrenaline are different enough to admit of a differential estimation of the two amines by measuring the fluorescence intensity at different activating wave-lengths (CARLSSON, ROSENGREN, BERTLER and NILSSON, 1957, BERTLER, CARLSSON and ROSENGREN, 1958). Dopamine behaves much like adrenaline and noradrenaline (DULIÈRE and RAPER, 1930), and the trihydroxyindole method, which utilizes this principle, can be modified to become a sensitive and specific method for the detection and assay of dopamine in the presence of adrenaline and

noradrenaline by utilizing differences in fluorescence characteristics (CARLSSON and WALDECK, 1958).

In order to make the fluorescence method suitable for a highly specific and accurate estimation procedure, the elimination of interfering substances and fluorescent contaminants is required.

The adsorption of catechol compounds onto aluminium hydroxide or aluminium oxide has been the current method for the isolation of adrenaline and noradrenaline from plasma (LUND, 1950, WEIL-MALHERBE and BONE, 1952, PRICE and PRICE, 1957, COHEN and GOLDENBERG, 1957). Apparently the contaminants, however, are not completely removed by the usual adsorption techniques. Increased specificity may be achieved through the elimination of interfering substances prior to the adsorption procedure.

According to the method of BERTLER, CARLSSON and ROSENGREN (1958) the proteins in tissue extracts are precipitated with perchloric acid prior to the separation of catechol amines by quantitative ion exchange method with a strong cation exchange resin, Dowex 50. This technique of purification and separation can be applied with good results to the estimation of catechol amines in plasma (BERTLER, CARLSSON, LINDQVIST and MAGNUSSON, 1958, VENDSALU, 1959). The initial precipitation step with perchloric acid leaves but minimal amounts of protein, largely mucoproteins, in the filtrate of plasma (WINZLER, DEVOR, MEHL and SMYTH, 1948, WEIMER and QUINN, 1958). The recovery figures obtained with this technique of deproteinization with subsequent separation of catechol amines by means of cation exchange resin appear to compare favourably with those obtained with other adsorbants, employed for the purpose of purification.

The improvement of the specificity of the trihydroxyindole procedure based upon the technique presented above appears to improve the procedure to the point where the recoveries are just as satisfactory as the specificity achieved.

NATELSON, LUGOVOY and PINCUS (1949) found that catechol amines formed strongly fluorescent condensation products with ethylenediamine. WEIL-MALHERBE and BONE (1953) applied this method to plasma extracts purified by alumina adsorption and obtained stable fluorescent products. By measuring the fluorescence of the condensation products at two wave-lengths with the use of differential secondary filters, the concentration of adrenaline and noradrenaline was determined. Concentrations of 1—1.5 μg adrenaline and 5.2 μg noradrenaline per litre in peripheral venous plasma were reported. Since then various modifications have been reported (for literature, see WEIL-MALHERBE, 1959,

MANGER, WAKIM and BOLLMAN, 1959). The values reported do not establish a satisfactory correlation with bioassays (WEIL-MALHERBE and BONE, 1957). The specificity of the ethylenediamine condensation method has been questioned by some workers (HOLZBAUER and VOGT, 1954, EULER and FLODING, 1955, VALK and PRICE, 1956, COHEN and GOLDENBERG, 1957).

Chapter II

Method

The method for the quantitative determination of adrenaline and noradrenaline in plasma described below is principally based on the method for the fluorometric determination of these amines in tissues worked out by BERTLER, CARLSSON and ROSENGREN (1958). The original method was also applied for chemical determination of catechol amines in the plasma of experimental animals (BERTLER, CARLSSON, LINDQVIST and MAGNUSSON, 1958). Modifications in technique in the method presented below are used with regard to the extremely low concentrations of adrenaline and noradrenaline present in peripheral venous plasma under normal physiologic conditions. A preliminary report dealing with some of the results of the present investigation was published by the Author in 1959.

Experimental

Reagents. Commercially available reagent grade chemicals were utilized without further purification. l-Ascorbic acid was obtained from Hoffmann-La Roche, Basel. Stock solutions of l-adrenaline base and of l-noradrenaline bitartrate monohydrate (Rhône-Poulenc, Paris) were prepared in 0.01 N hydrochloric acid at concentrations corresponding to 500 μg catechol amine base per ml.

For the preparation of all solutions only glass-distilled water was used. The necessity of using fresh reagents should be emphasized. All glassware was scrupulously cleaned, allowed to soak for at least one hour in dilute acid, and then rinsed with tap water and distilled water.

Apparatus. A standard Aminco-Bowman spectrophotofluorometer was used. Spectra were recorded on a cathode-ray oscillograph.

Preparation of Plasma Samples

For each determination an 18 ml sample of blood was collected in a cold, graded silicone-coated centrifuge tube containing 2 ml of 1 per cent disodium ethylenediaminetetraacetate (EDTA) in 0.9 per cent NaCl solution. The blood samples were mixed with the anticoagulant by repeated inversion of the tubes and the latter stored in ice-water.

The cold blood specimens were centrifuged as soon as possible, or within half an hour at the latest, at about $1000 \times g$ for 25 minutes in a refrigerated centrifuge at +4°C. As much as possible of the plasma solution was sucked off and the volume measured. It was then transferred to a centrifuge tube. The bulk of proteins was removed by precipitating with 0.1 volume of 4 N perchloric acid during brief stirring with a glass rod. The tube with solution and precipitate was then allowed to stand for 30 minutes at +4°C, and the liquid was next separated from the solid by centrifugation at about $1000 \times g$ for 10 minutes. The supernatants were withdrawn and the volumes measured. The pH of each specimen was adjusted to 4 with 5 N potassium carbonate using bromthymol blue (one drop of a 0.05 per cent solution in ethanol) as indicator, and the final adjustment was checked with indicator paper. The potassium carbonate was added dropwise from a 1.0 ml pipette into the centre of each sample and the tube was quickly shaken to obtain rapid distribution of the alkali. The potassium perchlorate was spun down at +4°C. The precipitate was washed with a few ml of glass-distilled water, and after centrifugation the supernatant was added to the plasma extract.

Comment: In order to preserve the ion exchange columns it is essential to obtain clear extracts.

The acid supernatants derived after deproteinization can be left in a freezing box. No destruction of catechol amines could be detected after a week, which is the longest time investigated.

Purification of Extracts

A Dowex 50—X4 column was used (200 to 400 mesh, dry weight 150 mg, dimensions 13 $mm^2 \times 35$ mm) in hydrogen form. Before the resin was used it was washed several times with 2 N hydrochloric acid. Glass-distilled water was then passed through the column until the pH of the effluent rose to about 5.

The plasma extract with pH adjusted to 4 was thereafter passed through the column. After rinsing with 50 ml of glass-distilled water, 6 ml of 0.1 M phosphate buffer pH 6.5 was passed through the column in order to remove interfering substances. Elution was performed with 4 ml of N hydrochloric acid. The rate of flow should be kept at 0.20 to 0.25 ml per minute. The rate of flow was easily controlled using the apparatus described by Bertler, Carlsson and Rosengren (1958).

The columns can be used for several determinations if they are washed with 40 ml of 2 N hydrochloric acid between the runnings.

Table 1. *Uptake of adrenaline (A), noradrenaline (NA), dopamine (DA) and dopa by a Dowex 50—X4 column (150 mg dry weight, dimensions 13 mm² × 35 mm, H^+ form) from a solution of sodium chloride and disodium ethylendiaminetetraacetate (EDTA) in about the same amounts as in the plasma sample.*

Elution was performed by means of 0.1 M phosphate buffer pH 6.5 and N hydrochloric acid followed by 2 N acid.

Added, µg	phosphate buffer 1—6 ml	Found, µg			
		N HCl		2 N HCl	
		1—4 ml	5—8 ml	1—5 ml	6—10 ml
A, 1.0	0.04	0.93	0.06	—	—
NA, 1.0	0.02	0.95	0.04	—	—
DA, 1.0	0.03	0.13	—	0.74	0.05
Dopa, 1.0	0.40	0	—	0	—

Adrenaline and noradrenaline are quantitatively found in the first fraction of eluate obtained with 4 ml of N hydrochloric acid and most of the dopamine in the second eluate, that with 6 ml of 2 N hydrochloric acid. When a solution containing dopa is applied to the column, the dopa is not retained quantitatively. During the wash with phosphate buffer such dopa as has been retained is eluted. As the precise amount retained will depend on various factors, such as the concentration of dopa or of inorganic ions, the exact amount of dopa retained may vary from one experiment to another.

Comment: The absorption of adrenaline and noradrenaline on the small ion exchange columns and the elution of these amines from the resins in hydrogen form have been studied in experiments with pure solutions (Table 1). The purification of tissue extracts as reported by BERTLER, CARLSSON and ROSENGREN (1958) was performed by means of cation exchange resin in sodium form. In present investigation satisfactory recoveries were obtained in experiments with ion exchange resin either in sodium or hydrogen form. The results of duplicate determinations, however, showed a closer agreement in our experiments, when the separation of catechol amines was performed with the resin in hydrogen form.

Eluates collected in small bottles may be frozen and stored therein for a week before analysis, without significant loss of catechol amines.

Assay of Catechol Amines

The adrenaline and noradrenaline in the eluates were determined spectrophotofluorometrically after oxidation and subsequent rearrange-

Table 2. *Example of analysis of adrenaline (A), and noradrenaline (NA) in an eluate from a blood sample drawn from the antecubital vein.*

	Sample	Faded blank	A, standard	NA, standard	Reagent blank
NA*, ml	—	—	—	0.20	—
A*, ml	—	—	0.20	—	—
H_2O, ml	0.30	0.27	2.10	2.10	2.30
Phosphate buffer, ml	0.30	0.30	0.30	0.30	0.30
Eluate, ml	2.00	2.00	—	—	—
$ZnSO_4$, ml	0.05	0.05	0.05	0.05	0.05
$K_3Fe(CN)_6$, ml	0.05	0.05	0.05	0.05	0.05
NaOH, ml	—	0.30	—	—	—
Ascorbic acid, ml	—	0.03	—	—	—
NaOH-ascorbic acid, ml	0.30	—	0.30	0.30	0.30
Fluorescence intensity at fluor. wave-length 540 mμ, arbitrary units					
Activating w.-l. 410 mμ	2.0 (S_1)	1.4	86.0 (A_1)	70.0 (NA_1)	1.0
Activating w.-l. 455 mμ	2.3 (S_2)	2.1	86.0 (A_2)	20.0 (NA_2)	1.8

*Containing 1.0 μg catechol amine base per ml.

ment to adrenolutine and noradrenolutine, respectively, essentially according to the procedure reported by BERTLER, CARLSSON and ROSENGREN (1958), principally based on the method of EULER and FLODING (1955).

After adjustment of pH to about 6.2—6.4 by means of 5 N potassium carbonate, the correct end point being checked with indicator paper, an eluate fraction of 2 ml was transferred to a centrifuge tube for assay. 0.3 ml of 0.1 M phosphate buffer, pH 6.5, 0.05 ml of zinc sulfate solution (0.5 g $ZnSO_4 \cdot 7\ H_2O$ in 100 ml water) and sufficient glass-distilled water to give a final volume of 3 ml were added. Oxidation was performed by addition of 0.05 ml of potassium ferricyanide solution (0.25 g $K_3Fe(CN)_6$ in 100 ml water). After 5 minutes, 0.3 ml of a combined reagent consisting of 9 parts of 5 N sodium hydroxide mixed immediately before use with 1 part of a freshly prepared 2 per cent aqueous solution of ascorbic acid was added. On addition of ascorbic acid the ferricyanide is reduced and the solution becomes colourless. The rearrangement to the fluorescent compound is completed in 5 minutes. Samples were mixed thoroughly by gently tapping the side of each tube with the

forefinger. In order to remove a slight cloudiness the samples were then centrifuged at about $1000 \times g$ for 5 minutes. This left a perfectly clear supernatant which is essential for the fluorometric estimation. Faded blanks were used, *i.e.* samples in which the fluorescent product had been allowed to develop and fade. Blanks were treated in the same way as the samples except that ascorbic acid was added 5 minutes after the sodium hydroxide. Standards of adrenaline and noradrenaline as well as reagent blank were run parallel with the samples. See Table 2.

Comment: The adjustment of pH to 6.2—6.4 should be carried out carefully and no more than four samples should be handled at one time. If the pH is allowed to increase above 7.5, the sample should be discarded. The pH adjustment of the samples should be finished within 10 minutes and the oxidation procedure begun immediately thereafter.

To evaluate possible interfering substances, known amounts of adrenaline or noradrenaline were added to a fraction of eluate, which was then treated in the same way as the sample ("internal standards"). As the obtained figures showed a close agreement with the expected ones the "internal standards" were not run as a routine.

In view of the low concentrations of catechol amines in plasma, all steps in the procedure have to be very carefully standardized.

Spectrophotofluorometric Differentiation of Adrenaline and Noradrenaline

The activation peaks of adrenolutine and noradrenolutine are at 425 and 410 mμ, respectively, the corresponding fluorescence peaks being at 545 and 535 mμ, respectively. (All wave-lengths given being uncorrected instrumental values.) The activation spectra of adrenolutine and noradrenolutine are sufficiently separated to allow a differential estimation of the two amines by measuring the fluorescence intensity at different activating wave-lengths (*e.g.* 410 and 455 mμ). Readings were performed at a fluorescent wave-length of 540 mμ (see BERTLER, CARLSSON and ROSENGREN, 1958).

Amounts ranging from 0.002 to 0.09 μg of adrenaline and 0.002 to 0.098 μg of noradrenaline were added to a solution containing sodium chloride, disodium ethylenediaminetetraacetate and perchloric acid in about the same amounts as in the plasma sample. The proportions of added catechol amines ranged from 1:1 to 49:1. A reasonably good agreement between expected and observed values was obtained (Table 3).

Calculation of Results

The quantities of adrenaline and noradrenaline present are calculated by means of the following system of simultaneous equations:

$$\frac{x \cdot A_1}{0.2} + \frac{y \cdot NA_1}{0.2} = S_1 \quad (1)$$

$$\frac{x \cdot A_2}{0.2} + \frac{y \cdot NA_2}{0.2} = S_2 \quad (2)$$

In these equations, x and y are the amounts of adrenaline and noradrenaline in μg in the sample, respectively; S_1 and S_2 are the fluorescence readings for the sample at activating wave-length 410 and 445 mμ, respectively, (corrected for the fluorescence of the faded blank); and A_1, A_2, NA_1 and NA_2 are the values of the fluorescence readings at activating wavelength 410 and 455 mμ for adrenaline and noradrenaline standards (*in casu*: 0.2 μg), respectively, corrected for the reagent blanks. See Table 2.

Elimination of x in (1) and (2) yields:

$$\text{noradrenaline (μg per sample)} = y = \frac{0.2(A_2 \cdot S_1 - A_1 \cdot S_2)}{A_2 \cdot NA_1 - A_1 \cdot NA_2} \quad (3)$$

x is subsequently determined by the formula:

$$\text{adrenaline (μg per sample)} = x = \frac{0.2 \cdot S_1 - y \cdot NA_1}{A_1} \quad (4)$$

which follows directly from (1).

Recovery Experiments

Amounts of adrenaline and noradrenaline ranging from 0.002 to 1.0 μg were added in various proportions to forty-seven 10 ml samples of human plasma prior to deproteinization. The pH adjusted plasma extracts were then passed through the ion exchange columns, eluted and analyzed as described above. The results were compared with those obtained from plasma samples to which no amines had been added, and the recoveries calculated. The overall mean recoveries for adrenaline and noradrenaline were 80.2 and 83.0 per cent, respectively, with standard deviations of 13.1 and 11.4, respectively. The increased variation in recoveries observed when small additions were made to plasma was of about the same order as the variation in replicates of a single sample.

Table 3. *Differential estimation of adrenaline (A) and noradrenaline (NA).*
Activating wave-lengths 410 and 455 mμ. Fluorescent wave-length 540 mμ.

μg added		μg found		per cent	
A	NA	A	NA	A	NA
0.002	0.098	0.0021	0.0945	105	94
0.005	0.095	0.0058	0.0941	116	99
0.010	0.090	0.0094	0.0882	94	98
0.020	0.080	0.0185	0.0784	92	98
0.030	0.070	0.0275	0.0686	92	98
0.050	0.050	0.0495	0.0471	99	92
0.005	0.005	0.0044	0.0047	88	94
0.080	0.020	0.0796	0.0200	99	100
0.090	0.010	0.0871	0.0117	97	117
0.002	0.002	0.0019	0.0020	95	100

Table 4. *Recovery of added adrenaline (A) and noradrenaline (NA) to human plasma.*

Added A and NA (μg/10 ml plasma)		Recovery (%)			
		A Mean S.D.		NA Mean S.D.	
I	0.002—0.01	n=10	81.6±21.4	n= 9	84.6±19.6
II	0.02 —0.3	n=16	74.4±10.7	n=14	78.6± 9.3
III	0.4 —1.0	n=21	83.9± 7.6	n=23	85.1± 7.7
IV	0.002—1.0	n=47	80.2±13.1	n=46	83.0±11.4

It thus probably represents the variation in the analytical method rather than variation in the recovery (Table 4). This becomes apparent when low concentrations of adrenaline and noradrenaline are analyzed.

Error of the Method

In order to test the accuracy of the method, each one of forty 20 ml plasma samples was divided in two, and each fraction was separately treated according to the procedure described above. For the assay of adrenaline, with a mean concentration of 0.17 μg per litre, the standard deviation was 11.8 per cent. For noradrenaline, with a mean concentration of 0.39 μg per litre, the standard deviation corresponded to 9.6 per cent. The blood samples were derived from different sites of blood

flow in the body, and the calculated values of adrenaline and noradrenaline ranged from −0.04 to 0.54 and from 0.08 to 2.64 μg per litre of plasma, respectively. It must be realized that, when determinations are made of very small amounts of catechol amines, *e.g.* the plasma concentrations derived from peripheral venous blood under normal physiological conditions, the procedure is less accurate than is the case when larger concentrations are analyzed.

Determination of Dopamine

The preparation of samples and the purification procedure were performed as described above. Adrenaline and noradrenaline were quantitatively found in the first portion of eluate, that of 4 ml of N hydrochloric acid, and most of the dopamine in the second eluate, that of 6 ml of 2 N hydrochloric acid. Oxidation of dopamine in the eluate was carried out as reported by CARLSSON and WALDECK (1958). The fluorophore of dopamine obtained by oxidation and rearrangement in alkali has activation and fluorescence peaks at 345 and 410 mμ, respectively, (uncorrected instrumental values).

Comment: The method was used only in a few experiments for the determination of dopamine in plasma derived from venous blood of healthy subjects, and in cases of phaeochromocytoma.

Discussion of the Specificity and Sensitivity of the Method

As observed by LUND (1949) and concluded on theoretical grounds by BU'LOCK and HARLEY-MASON (1953), the structural requirements for the formation of a fluorescent compound in the trihydroxyindole reaction appear to be a side chain of special configuration attached to the catechol nucleus. Apparently the side chain must contain a hydroxyl group at the β-carbon (next to the ring) and hydrogen at the α-carbon and terminate with an amino or monoalkyl-substituted amino group in order for fluoroscence to occur.

Adrenaline and noradrenaline are easily oxidized by potassium ferricyanide to the red indole derivatives, which in the absence of oxygen in alkali undergo intramolecular rearrangement to form the strongly fluorescent trihydroxyindoles with characteristic spectral properties. Catechol compounds possessing ethylamine or β-substituted alanine side chains, *e.g.* dopamine and dopa (3,4-dihydroxyphenylalanine) respectively, or compounds possessing ethanolamine side chains, *e.g.*

isopropylnoradrenaline *N*-isopropylethanolamine side chain), represent two types of potentially interfering substances. If dopamine is treated in this way, the fluorescence obtained is weak, with activation and fluorescence peaks indistinguishable from those of noradrenolutine (EULER and FLODING, 1955, SHORE and OLIN, 1958, CARLSSON and WALDECK, 1958). Dopa, when treated as above, yields a fluorescent compound with spectral characteristics different from those of adrenolutine and noradrenolutine (BERTLER and ROSENGREN, 1960).

Furthermore, using the quantitative ion exchange method described (see Table 1), dopa, adrenaline together with noradrenaline, and dopamine are satisfactorily separated from each other. Isopropylnoradrenaline probably does not represent any significant source of error, as the presence of this compound has not been demonstrated in man. 3-Methoxy analogues of adrenaline and noradrenaline are not as easily oxidized as the corresponding catechol amines, and potassium ferricyanide in the concentration employed for the oxidation of adrenaline and noradrenaline cannot be used for the preparation of the fluorophores of these 3-O-methylated derivatives (BERTLER, CARLSSON and ROSENGREN, 1959).

Some stable form of control is required to distinguish the fluorescence of the trihydroxyindoles from that of the contaminants. The use of faded samples, *i.e.* samples in which the fluorescent product has been allowed to develop and fade before addition of ascorbid acid has been found to be appropriate for this purpose. The major contribution to the residual fluorescence in such a "blank" (and sample) comes apparently from the products of decomposition of ascorbic acid in alkali. From tests in which the fluorescence is measured at intervals after addition of ascorbic acid to a solution containing the reagents (potassium ferricyanide and alkali) employed in the determination, the following facts were established. Fluorescence increases rapidly during the first 30 seconds, reaching a maximum within 1 minute; it then decreases slowly but after 5 minutes no further change is observed. In order, therefore, to obtain comparable values no readings were taken for 5—10 minutes after addition of the ascorbic acid either to the test sample or to the "blank" or control, which had first been allowed to fade by adding alkali alone instead of as a mixed solution containing ascorbic acid.

Experimental indication of the specificity of the trihydroxyindole method is afforded by the results obtained during intravenous infusions of catechol amines. The findings indicate the ability to differentiate between adrenaline and noradrenaline (see Chapter IV).

Summary

Trihydroxyindole fluorescence has been applied to the detection of adrenaline and noradrenaline in human plasma. In order to improve both the sensitivity and specificity of this quantitative chemical method, spectrophotofluorometry has been used for the assay. Simultaneous examination of both the activation and the fluorescence spectra provides powerful criteria for evaluating the specificity of the determination and for detecting the presence of interfering substances. The initial deproteinization prior to the separation of catechol amines by ion exchange appears to be an improvement, since it permits the preparation of sample blanks having weak and reasonably stable fluorescence.

Statistics

The procedure described by DAVIES (1949) was followed in the statistical treatment of the data obtained in the present investigation. P values less than 0.025 were considered to indicate significance.

Chapter III

Effect of Insulin Hypoglycemia on Plasma Concentrations of Adrenaline and Noradrenaline in Normal Subjects

The following study formed part of an investigation into the application of the spectrophotofluorometric method for the estimation of adrenaline in blood, and was undertaken partly as a test of the method in order to determine whether an increase in the adrenaline level in peripheral venous blood during insulin hypoglycemia could be demonstrated.

An increase in circulating adrenaline during hypoglycemia after administration of insulin has been shown in experimental animals (CANNON, MCIVER and BLISS, 1924, ABE, 1924, HOUSSAY, MOLINELLI and LEWIS, 1924, DUNÉR, 1953). POLL (1925) has shown depletion of the chromaffin substance of suprarenal medulla and HÖKFELT (1951), OUTSCHOORN (1952) and UDENFRIEND, COOPER, CLARK and BAER (1953) have demonstrated the decrease in the stores of adrenaline in the suprarenal medulla after administration of insulin. Hypoglycemia induced by intravenous administration of a standard dose of insulin to healthy human subjects has been shown to cause a large increase in the urinary excretion of adrenaline (EULER and LUFT, 1952, FRENCH, KILPATRICK and WOOD, 1955, ELMADJIAN, *et al.*, 1956).

HOLZBAUER and VOGT (1954) utilizing a specific method based on paper chromatographic separation of catechol amines from plasma, followed by biological assay, demonstrated an increase of the adrenaline level in blood from dogs and in one human subject after insulin injection. Using a fluorometric technique, WEIL-MALHERBE and BONE (1952, 1954) have reported a decrease in the concentration of plasma adrenaline during insulin-induced hypoglycemia. More recently, however, MILLAR (1956) has found increase in concentration of plasma adrenaline during hypoglycemia when a modification of the fluorometric method of Weil-Malherbe and Bone was used.

Material and Methods

The insulin response was studied in 31 healthy subjects (medical students and hospital employees), 18 males and 13 females, aged between 19 and 68.

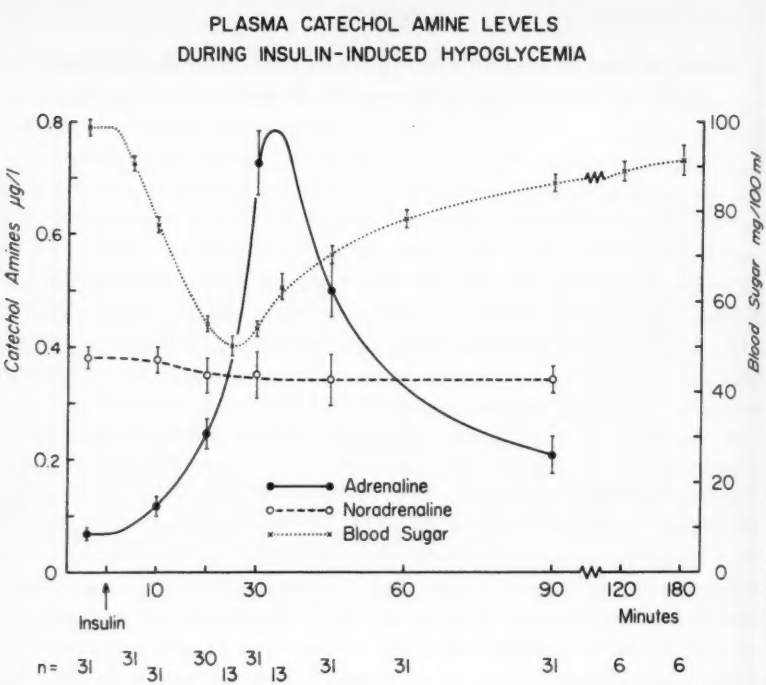


Figure 5.

The subjects were studied in the morning following an overnight fast. Each subject was allowed to rest for about 15 minutes following which a Cournand needle was inserted into the right antecubital vein for the withdrawal of blood samples. At 9 a.m., following withdrawal of the control sample of blood, insulin was injected intravenously in a dose of 0.1 I.U. per kilogram of bodyweight. Capillary blood samples for the determination of the blood sugar level were taken 5, 10, 20, 25, 30, 45, 60, 90, 120 and 180 minutes after the injection. The pulse rate and blood pressure were determined at regular intervals of 5 to 10 minutes. The subjects' reports on different symptoms of hypoglycemia were noted.

The blood samples for the determination of catechol amines were taken during the control period, and 10, 20, 30, 45 and 90 minutes after the administration of insulin.

Results

During the action of insulin, simultaneously with the most marked blood sugar decrease, there was a significant increase in pulse rate, a slight increase in systolic blood pressure and a fall of the diastolic pressure, the changes representing the characteristic circulatory responses to adrenaline. The subjects showed at the same time typical hypoglycemic symptoms of varying degree.

The mean concentrations of adrenaline and noradrenaline during the control period were 0.07 ± 0.01 μg and 0.38 ± 0.02 μg per litre of plasma, corresponding with the normal range of such values (see Table 7). Ten minutes after the administration of insulin the mean concentration of adrenaline was 0.12 ± 0.02 μg, twenty minutes after administration it was 0.25 ± 0.03 μg and thirty minutes after administration 0.73 ± 0.06 μg per litre of plasma. At this time the mean values of adrenaline concentration were highest. Forty-five minutes after administration of insulin the mean concentration of adrenaline had declined to 0.50 ± 0.05 μg per litre of plasma, and after ninety minutes the value was 0.21 μg per litre of plasma. The mean concentrations for noradrenaline were practically the same during the whole period of insulin-induced hypoglycemia. The mean concentration of adrenaline ten minutes after administration of insulin was not significantly increased in comparison with the resting value ($p > 0.05$), while the mean concentrations obtained 20, 30, 45 and 90 minutes after administration of insulin were significantly increased ($p < 0.001$). The results are shown in Figure 5 and further details are supplied in Table 1 and 2 in the Appendix.

Summary

The results obtained by the present method of estimating adrenaline and noradrenaline confirm and extend the findings of the previous workers referred to above. It may be seen that the sequence of changes in the blood sugar level are followed almost instantaneously by complementary changes in the level of adrenaline, while, as expected the level of noradrenaline did not alter to any marked extent. It was accordingly felt that the method would be reliable in other investigations.

Chapter IV

Plasma Concentrations of Adrenaline and Noradrenaline in Normal Subjects

The constant presence in urine of catechol amines in healthy subjects under normal circumstances indicates that these amines are probably continuously released in the organism. Since both adrenaline and noradrenaline are present not only in the suprarenals but noradrenaline also in the adrenergic nerves, and since at least adrenaline, is believed from indirect evidence to arise from scattered chromaffin cells outside the suprarenals, it is of interest to find how the proportions of the two amines vary in blood samples taken from different parts of the circulation.

Adrenaline and noradrenaline in the plasma taken from different points in the vascular system of the body were determined by use of the spectrophotofluorometric method.

Association of Adrenaline and Noradrenaline with Platelets

In the fractionation of the blood, the degree of force of the centrifuging could be of importance since it would affect the content of blood platelets in plasma and, inasmuch as platelets are reported to contain adrenaline and noradrenaline, indirectly also the concentration of adrenaline and noradrenaline in plasma (WEIL-MALHERBE and BONE, 1954, 1958).

Studies were undertaken to estimate the possible association of catechol amines with platelets, and to determine whether or not the overall concentration of catechol amines would be influenced by their presence or absence.

Methods

Platelet counts were made by the method of BJÖRKMAN (1959) on venous blood collected in silicone-coated tubes containing 1 per cent disodium ethylenediaminetetraacetate (EDTA) in 0.9 per cent NaCl solution. The counts were performed in duplicate.

Platelet-rich plasma was obtained by withdrawing blood, (36 ml) by the silicone technique into 4 ml of 1 per cent EDTA solution and then immediately centrifuging at $150 \times g$ at $+4°$ C for 12 minutes. The platelet-rich supernatant was thoroughly pipetted off.

Platelet-poor plasma was prepared by centrifuging platelet-rich plasma at $16000 \times g$ at $+4°$C for 50 minutes and the supernatant, poor in platelets, was carefully pipetted off.

Platelet suspensions were prepared from 200 ml of blood to which was added the required amount of 1 per cent EDTA solution. The centrifugation was performed as described above, and 10 ml samples of the platelet-rich and platelet-poor supernatant solutions were removed for determination of the catechol amine content. The platelets were washed twice with a solution consisting of one part of 1 per cent EDTA solution in 0.9 per cent NaCl and nine parts of distilled water, and were reprecipitated by centrifugation at $16000 \times g$ for 25 minutes. The washed platelets were resuspended in 10 ml of 0.9 per cent NaCl solution, and platelet counts were made.

Results

The concentrations of adrenaline and noradrenaline in platelet-rich and platelet-poor plasma samples were the same within the limits of error, in this series of 23 determinations (Table 5). It may be seen that there was no statistical difference between the values obtained from platelet-rich and platelet-poor plasma.

In order to test the problem more thoroughly, direct determination of the catechol amine concentrations in 6 preparations of platelets was carried out. As described above, the platelets were washed twice to remove plasma. This process is not considered to have removed catechol amines from the platelets, since the values obtained (Table 6) were unchanged after a further six similar washings. To facilitate comparison with the results of other authors, the concentrations were expressed as $m\mu g/10^9$ platelets. However, in order to compare the concentration in platelets with that in plasma, it is necessary to calculate the number of platelets per ml of a preparation free of plasma. For this purpose the data of MAUPIN (1954), quoted by WEIL-MALHERBE and BONE (1958), were used. Maupin concluded that, for a count of 5×10^5 platelets/μl of plasma, the platelet volume is 0.85 % of the total. This is equivalent to a count of 5.9×10^{10} platelets/ml of a pure platelet preparation. The mean concentration of total catechol amines in platelets was therefore 10 mμg/ml, a value some 16 times higher than that in platelet-poor

Table 5. *Distribution of adrenaline (A) and noradrenaline (NA) in platelet-rich and platelet-poor plasma in a series of 23 experiments.*

	Platelet-rich plasma mμg/ml Mean±S.E.	Platelet-poor* plasma mμg/ml Mean±S.E.	Diff.±S.E.
A	0.12±0.03	0.13±0.02	−0.01±0.02 p>0.5
NA	0.61±0.05	0.58±0.04	0.03±0.02 p>0.1
	n=23	n=23	

* This indicates a preparation containing less than 10^3 platelets/ml

Table 6. *Distribution of adrenaline (A) and noradrenaline (NA) in plasma and platelets.*

	Platelet-rich plasma mμg/ml Mean±S.E.	Platelet-poor* plasma mμg/ml Mean±S.E.	Diff.±S.E.	Platelets mμg/10^9 platelets Mean±S.E.
A	0.12±0.05	0.11±0.04	0.02±0.02 p>0.25	0.08±0.02
NA	0.50±0.05	0.49±0.07	0.01±0.05 p>0.5	0.08±0.02
Total catechol amines	0.64±0.07 n=6	0.61±0.06 n=6	0.03±0.07	0.17±0.04 n=6

* This indicates a preparation containing less than 10^3 platelets/ml

plasma. The mean platelet count in the platelet-rich plasma samples (Table 6) was $2 \cdot 34 \times 10^5$/ml; there would therefore have been approximately 0.07 mμg of catechol amines associated with platelets per ml of platelet-rich plasma. Since this is of the same order as the standard error calculated for the total catechol amine concentrations in platelet-rich plasma it is clear that no significant decrease would be expected by removal of this quantity of platelets, and such was indeed found to be the case, as may be seen from the values of p in the penultimate column of Table 6.

Concentrations of Adrenaline and Noradrenaline in Peripheral Venous Plasma

Material and Methods

After an ordinary Cournand needle had been introduced into the antecubital vein the subjects were allowed to rest in a supine position for about 10 minutes before the blood samples were collected directly into the centrifuge tubes. Blood samples were obtained from 125 healthy subjects (medical students and hospital employees). Seventy-five samples were obtained from males and fifty from females aged between 17 and 55 and between 17 and 68, respectively. Most of them were in their twenties.

Results

The mean, range, standard deviation and standard error of the mean concentration of adrenaline and noradrenaline are recorded in Table 7. Plasma concentrations ranged from -0.08 to 0.22 μg adrenaline and from 0.16 to 0.64 μg noradrenaline per litre. Small negative values for adrenaline were within the range of precision of the method. The following mean values and standard deviations were calculated: 0.07 ± 0.06 μg adrenaline and 0.35 ± 0.09μg noradrenaline per litre of plasma for males, and 0.06 ± 0.05μg adrenaline and 0.35 ± 0.10μg noradrenaline per litre of plasma for females. Thus there was no difference between the plasma concentration of catechol amines in the males and females. No relationship between age and concentration of catechol amines was apparent.

Arterio-Venous Differences in the Adrenaline and Noradrenaline Content of Plasma

Material and Methods

Blood samples were withdrawn from the antecubital vein and brachial artery of 29 healthy subjects. After local anaesthesia the artery was punctured and the blood was drawn into the syringe, the sample was thereafter transferred to a centrifuge tube.

In two subjects an ordinary Cournand needle was introduced into the antecubital vein and a polyethylene catheter into the brachial artery according to the technique described by HOLMGREN (1956). The subjects were allowed to rest for fifteen minutes and the blood samples were taken after that. Blood samples from the antecubital vein and brachial artery were taken simultaneously at five-minute intervals, four times in all.

Table 7. *Concentration (μg/litre) of adrenaline (A) and noradrenaline (NA) of peripheral venous plasma of 125 healthy subjects.*

	Males		Females	
	A	NA	A	NA
Mean	0.07	0.35	0.06	0.35
S.E.	±0.01	±0.01	±0.01	±0.01
S.D.	±0.06	±0.09	±0.05	±0.10
n	75		50	

Table 8. *Arterio-venous differences in the adrenaline (A) and noradrenaline (NA) content (μg/litre) of plasma in 29 healthy subjects*

	Brachial artery		Antecubital vein		Arterio-venous diff.	
	A	NA	A	NA	A	NA
Mean	0.23	0.31	0.07	0.40	0.16	—0.09
S.E.	±0.02	±0.02	±0.01	±0.02	±0.02 $p<0.001$	±0.02 $p<0.001$

Results

The concentration of adrenaline in arterial plasma of 29 healthy subjects averaged 0.23 μg per litre, while the concentration in venous plasma averaged 0.07 μg per litre. The difference of 0.16 μg per litre plasma was statistically significant ($p<0.001$).

The concentration of noradrenaline in venous plasma exceeded that in arterial plasma. The average concentration of noradrenaline in venous and arterial plasma was 0.40 μg and 0.31 μg per litre, respectively. The difference in concentration averaged 0.09 μg per litre, which was statistically significant ($p<0.001$). See Table 8.

In the two normal subjects whose blood was obtained through a needle some time after it had been placed in the vein in order to reduce all extraneous sensory stimuli to a minimum, the average concentration of adrenaline in arterial and venous plasma was 0.40 and 0.10 μg per litre plasma, respectively. The average noradrenaline concentration in arterial and venous plasma was 0.28 and 0.46 μg per litre, respectively. The differences were statistically significant ($p<0.001$). See Table 9.

A positive arterio-venous difference for adrenaline implies a continuous removal of adrenaline in the peripheral tissues. Assuming an average

plasma volume of 3.6 litres (based on an average blood volume of 6 litres, a haematocrit of 40, and a mean body weight of 70 kg) the rate of utilization of adrenaline may be calculated as 0.008 μg/kg/min. The value obtained here agrees with that calculated by COHEN, HOLLAND, SHA and GOLDENBERG (1959) from steady state concentrations resulting from infusions into healthy human subjects. Similar infusion studies carried out in the course of the present work (see Chapter V) gave a similar value. The corresponding value, obtained from the data for the negative arterio-venous difference in the case of noradrenaline, is 0.005 μg/kg/min. This value is an indication of the minimum rate of production or release of this substance in the peripheral tissues; the true value may be substantially higher depending on the rate of simultaneous metabolic destruction or removal of noradrenaline in the peripheral tissues.

Results Obtained by Heart Catheterization

Material and Methods

Blood samples were obtained from subjects submitted to diagnostic cardiac catheterization. In 14 subjects normal findings were encountered and the presence of a normal cardiovascular system was established (Table 3, I in the Appendix). In 16 subjects the diagnosis of organic heart disease was confirmed. The right heart catheterization in this group revealed normal pressure findings. Ten of these subjects had mitral, tricuspid or aortic valvular disease, while six subjects had simple atrial or ventricular septal defects (Table 3, II, a and b in the Appendix). Finally 29 subjects had dynamically significant heart disease with elevated pressure findings at right-heart catheterization (Table 3, III in the Appendix).

In practice, the catheter was advanced from an antecubital or a saphenous vein and its tip was lodged at the most distal point desired, either in the lower part of the inferior vena cava or in the pulmonary artery. A series of blood samples was obtained by withdrawal of blood *via* the catheter from different points in the vascular tree as the tip of the catheter was withdrawn to these sites in rapid sequence under fluoroscopic control. The specimens were drawn approximately at three-minute intervals in the course of a single passage up or down the vena cava. In some subjects the catheter was thereafter introduced into the hepatic or renal veins. This was sometimes rather a time-consuming procedure. Blood samples from the antecubital vein or the femoral artery were obtained through a needle, and a local anesthetic agent was introduced

Table 9. *Arterio-venous differences in the adrenaline (A) and noradrenaline (NA) content of plasma in two healthy subjects at rest* (μg/l).

In each case four blood samples were obtained from brachial artery and four samples from antecubital vein with 5 minutes intervals.

Brachial artery		Antecubital vein		Arterio-venous diff.	
A	NA	A	NA	A	NA
0.38	0.29	0.16	0.41	0.22	—0.12
0.38	0.29	0.15	0.48	0.23	—0.19
0.48	0.30	0.15	0.49	0.34	—0.19
0.44	0.28	0.14	0.45	0.30	—0.17
0.37	0.28	0.06	0.50	0.31	—0.22
0.41	0.30	0.06	0.43	0.35	—0.13
0.35	0.27	0.06	0.47	0.29	—0.20
0.37	0.21	0.06	0.46	0.31	—0.25
Mean±S.E. 0.40±0.02	Mean±S.E. 0.28±0.01	Mean±S.E. 0.10±0.02	Mean±S.E. 0.46±0.01	Diff.±S.E. 0.29±0.02 $p<0.001$	Diff.±S.E. —0.18±0.02 $p<0.001$

around the artery to be punctured. The blood samples were transferred to silicone-coated centrifuge tubes, which were stored in ice-water until the procedure was completed, or at most for half an hour.

Results

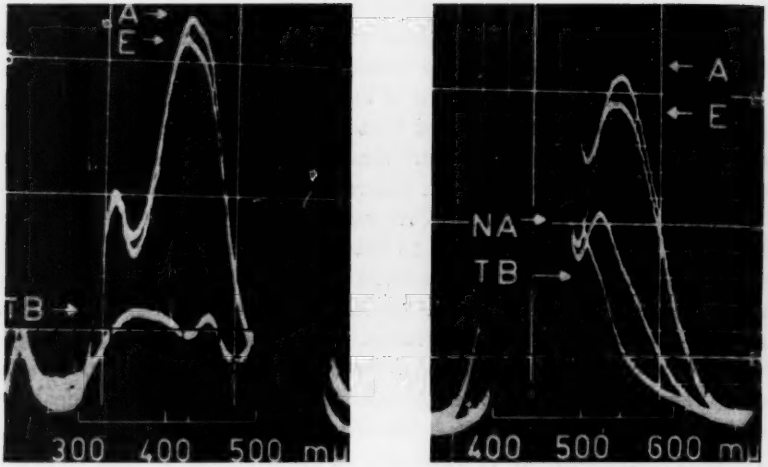
It was not the purpose of the present work to draw conclusions from the relatively small differences between these different groups of normal or diseased subjects, since these differences were usually no larger than the differences observed when the site of withdrawal of blood was altered slightly. It will, however, be clear that if significant differences of this sort, between healthy and diseased people, are to be established, then the precise site of blood sampling will have to be carefully controlled. Results obtained from studies of patients are included for purposes of comparison, and to demonstrate the marked way in which the concentrations depend, in all groups, on the position of the tip of catheter.

The mean, range and standard error of the mean concentrations of adrenaline and noradrenaline appear in Table 3, in the Appendix. The mean concentrations of adrenaline in the antecubital vein varied from 0.05 to 0.14 μg and the mean concentrations of noradrenaline from 0.32 to 0.36 μg per litre of plasma in different groups.

The highest concentration values of adrenaline were found in the samples obtained from the left renal vein, which usually receives blood from the suprarenal gland. The mean value of adrenaline in the left renal vein of the group consisting of eight healthy subjects was 0.36 μg per litre of plasma, while that of noradrenaline was 0.18 μg. The mean concentration values found in subjects with heart disease ranged from 0.32 to 3.14 μg of adrenaline in different groups, while the mean concentration values of noradrenaline ranged from 0.27 to 0.92 μg per litre. The adrenaline amounted to between 54 and 87 per cent of the total catechol amines in the left renal vein. The activation and fluorescence spectra recorded argued for the presence of adrenaline and a smaller proportion of noradrenaline. See Figure 6. Relatively high values of adrenaline were noted in the upper part of the inferior vena cava; the mean concentration of adrenaline in the group of healthy subjects was 0.39 μg per litre, while that of noradrenaline was 0.29 μg. The mean concentrations of adrenaline in the groups comprising subjects with heart disease ranged from 0.35 to 0.50 μg and of noradrenaline from 0.21 to 0.29 μg per litre. Even though in this case, the activation and fluorescence peaks could not be determined with any very high degree of accuracy, it is reasonable to assume that the comparatively high values in this part of the circulation are due to the presence of suprarenal venous blood and thus really represent adrenaline.

The values of noradrenaline were always higher than those of adrenaline except in the samples obtained from the left renal vein or the upper part of the inferior vena cava.

The lowest mean concentration values of adrenaline and noradrenaline were obtained in the samples derived from the hepatic vein and ranged from 0.03 to 0.09 and from 0.09 to 0.17 μg per litre, respectively, in the different groups. When the mean values of noradrenaline obtained from the hepatic vein were compared with those obtained from the antecubital vein, the differences were found to be statistically significant in the group of healthy subjects and in that of subjects with elevated pressure values found by right heart catheterization ($p<0.001$ and $p<0.025$, respectively). It should be mentioned that the concentration of total catechol amines in samples derived from the hepatic vein sometimes differed insignificantly from zero, which was never the case with samples derived from other sites in the vascular tree of the body.



I II

Figure 6. Activation and Fluorescence Spectra of an Eluate from a Blood Sample from the Left Renal Vein after Oxidation and Rearrangement in Alkali.

I Activation spectrum. Fluorescence wave-length 540 mμ.
II Fluorescence spectrum. Activating wave-length 455 mμ.
A Adrenaline standard.
NA Noradrenaline standard.
TB Faded sample blank.

The activation and fluorescence peaks obtained from adrenaline are 425 and 545 mμ, respectively. The corresponding peaks for noradrenaline are at 410 and 535 mμ, respectively. The spectra of the eluate come close to those obtained from adrenaline.

Discussion

From results obtained it can be stated that under normal physiological conditions the concentrations of adrenaline and noradrenaline in blood samples from most parts of the circulation in the human body are very low. The most sensitive and specific bioassay procedures fail to detect catechol amines in peripheral blood. Thus, in the experiments of Holzbauer and Vogt (1954) the concentrations of adrenaline and noradrenaline in plasma obtained from a resting subject were concluded to be less than 0.06 μg and 1.0 μg per litre plasma, respectively. It should be realized that if the trihydroxyindole method (or any other current chemical method available) is pushed to the limits of sensitivity, as must be done for

I II III

Figure 7. Activation and Fluorescence Spectra of an Eluate from a Blood Sample Obtained from Peripheral Vein after Oxidation and Rearrangement in Alkali.

I Activation spectrum. Fluorescence wave-length 540 mμ.
II Fluorescence spectrum. Activating wave-length 410 mμ.
III Fluorescence spectrum. Activating wave-length 455 mμ.
A Adrenaline standard.
NA Noradrenaline standard. Calculated value: 0.07 μg adrenaline and
TB Faded sample blank. 0.35 μg noradrenaline per litre of plasma

blood analyses, these limits will be governed to a greater extent by the fluorescence of the sample blank. Figure 7 illustrates representative activation and fluorescence spectra from a blood sample obtained from a peripheral vein; these spectra might well correspond with noradrenaline It is, however, evident from comparison of Figure 6,I with Figure 7,I that resolution is to some extent lost when the height of the sample peak is low and near to that of the control.

Although at first some doubts were felt about the suitability of this method for the determination of the minute amounts of the catechol amines in question, it would appear from the data of Table 9, and 3 in the Appendix that the method does in fact yield reproducible values. Table 9 gives data from such a serial sampling in two patients. Considering what has been said above it is reasonable to conclude that adrenaline is secreted into blood entering the upper part of the inferior vena cava and disappears in the peripheral tissue (positive arterio-venous difference). It is also not unreasonable to assume that noradrenaline is released by the adrenergic nerve endings. Finally, the lowest values obtained from blood samples from the hepatic vein suppor the hypothesis that the organs in the region of the portal circulation are able to metabolize catechol amines. It should, however, be pointed out that all these data have been obtained by using the method of estimation at its lower limits of accuracy; in order to obtain more convincing and reliable data it was necessary to raise the concentration of catechol amines by a technique such as that of continuous infusion. This phase of the investigation is described in the next chapter.

If it were assumed that the concentration of noradrenaline in hepatic venous blood is not significantly different from zero (see Table 3 in the Appendix), then the portal blood would be completely cleared of noradrenaline on reemerging in the hepatic vein and an approximate half-life corresponding to removal in this way may be calculated in the following manner.

The portal blood flow is approximately 1.2 l/min. (SHERLOCK, 1958) and the blood volume is about 6 litres. Therefore one fifth of the blood would be cleared of noradrenaline per minute, and this would be the value of the constant k in the following equation for disappearance by a first-order process of any substance having initial concentration X, and a concentration x after t minutes.

$$x = \mathrm{X} \cdot \mathrm{e}^{-kt}$$

The half-life, $t_{\frac{1}{2}}$, of x may then be calculated from the relationship,

$$t_{\frac{1}{2}} = \frac{\log_e 2}{k} = \frac{0.693}{0.2} = 3\tfrac{1}{2} \text{ minutes}$$

The hepatic arterial blood flow is reported to vary greatly (SHERLOCK, 1958). This calculation does not therefore take into account any clearance from the hepatic arterial blood which would lower the value of $t_{\frac{1}{2}}$. It should however be pointed out that, as the total renal blood flow is also about 1.2 l/min. a similar half-life would result if elimination were primarily effected by complete clearance from renal blood. It is possible to infer from the studies of EULER and LUFT (1951) that there is active tubular reabsorption of noradrenaline in the kidney, since these authors found that only 1.5 to 3.3 per cent of an infused amount was present in the urine, but it is still not known whether or not any noradrenaline reabsorbed from the glomerular filtrate returns to the general circulation. Such a problem could, of course, be solved by measuring the renal arterio-venous difference in noradrenaline concentration. If both the renal and the hepatic routes of elimination were operating, then $t_{\frac{1}{2}}$ would be about $1\frac{3}{4}$ minutes.

The approximate value of $t_{\frac{1}{2}}$ may then be compared with the values for $t_{\frac{1}{2}}$ calculated from the infusion studies in the next chapter. If approximate agreement is obtained it would suggest, when considered in conjunction with the low values rather frequently obtained for hepatic venous blood (Table 3 in the Appendix), that removal by the liver is an important factor causing disappearance of noradrenaline from the blood.

The values in peripheral venous plasma obtained in the present work, are in good agreement with those obtained by VALK and PRICE (1956), and COHEN and GOLDENBERG (1957), who use the trihydroxyindole procedure but much lower than those obtained by the ethylenediamine condensation method of WEIL-MALHERBE and BONE (1953).

The findings with respect to the relative concentrations of catechol amines in platelets and in plasma indicate that while the concentration in platelets is higher than in plasma, the contribution of platelet-bound amines to the total in platelet-rich plasma is insignificant. These results agree therefore with those of VALK and PRICE (1956) rather than with those of WEIL-MALHERBE and BONE (1954, 1958).

Chapter V

Plasma Concentrations of Adrenaline and Noradrenaline during Intravenous Infusions

Although the physiological and pharmacological effects of adrenaline and noradrenaline have been the subject of numerous clinical studies, the plasma concentrations attained by infusion of catechol amines have been investigated only in a few studies (MILLAR, 1956, PRICE, 1957, COHEN, HOLLAND, SHA and GOLDENBERG, 1959). Investigation of plasma adrenaline and noradrenaline levels during intravenous infusions was undertaken and the results are given below.

Experimental

Infusion studies were performed on a group of volunteers (medical students). Sterile saline solutions of l-adrenaline or l-noradrenaline (Adrenaline, "ACO", or Nor-Exadrin conc., "Astra") for infusion were prepared at concentrations of 0.2 or 4.0 μg of catechol amine base per ml. Each study was initiated with a ten-minute control period when a saline infusion was performed by means of intravenous drop. Thereafter the administration of adrenaline or noradrenaline began. Flow rates were controlled by continuous counting of drops per time-unit. Flow rates of 0.5 to 2.0 ml per minute were used.

A total of eleven infusions were performed. Subjects received infusion into the right antecubital vein while resting in a supine position. Blood pressure and pulse rate were noted at various intervals. Blood specimens were obtained through a needle lodged in the left antecubital vein and the blood was directly collected in the cold centrifuge tubes. In three experiments a Cournand catheter was advanced from a vein in the left forearm, and its tip was lodged in the hepatic vein. The blood samples were withdrawn and transferred to centrifuge tubes. Specimens were chilled in an ice-water bath. They were then centrifuged at $1000 \times g$ for 25 minutes, after which the supernatant plasma was removed and treated as for catechol amines described in Chapter II.

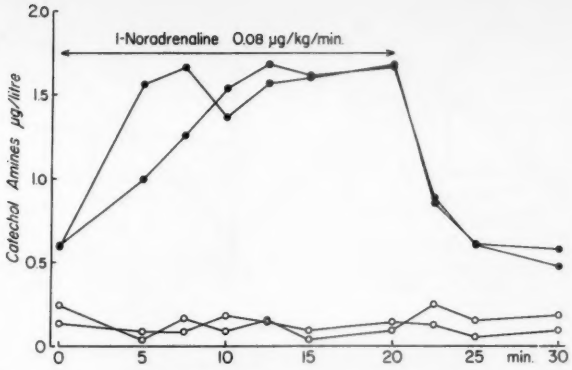


Figure 8. Peripheral venous plasma concentrations of noradrenaline observed during intravenous infusions at constant rate.
Normal male subject, J. Å., 22 years, 76 kg
Normal male subject, J. M., 25 years, 60 kg
The upper two curves represent levels of noradrenaline, the higher resting value being found in subject J. M. The lower two curves represent levels of adrenaline.

The highest infusion rate for noradrenaline was 0.1 µg/kg/min. and for adrenaline 0.21 µg/kg/min. Infusion was stopped at the subject's request, or if headache was reported, or if the subject appeared uncomfortable.

Results

Constant rate infusions of adrenaline or noradrenaline were characterized by the attainment of a steady state plasma concentration; for noradrenaline, see Figure 8. Although fluctuations in the steady state concentration were observed, the data were in general indicative of the maintenance of a balance between the rate at which the particular catechol amine was added to plasma and the rate at which it was simultaneously removed. Steady state concentrations were attained within 7½ to 10 minutes.

During the infusion of one catechol amine (either adrenaline or noradrenaline), the changes in the plasma concentration of the alternate non-infused catechol amine were small. Representative data may be found in Figures 9 and 10, where the results of stepwise infusions at successively higher rates are shown. Blood specimens were withdrawn at each infusion rate after a lapse of 15 minutes, *i.e.* at the steady state.

The steady state plasma concentrations of infused catechol amine were related linearly to the infusion rate; this is demonstrated in Figure 9 and 10 where the results of separate infusions of adrenaline and norad-

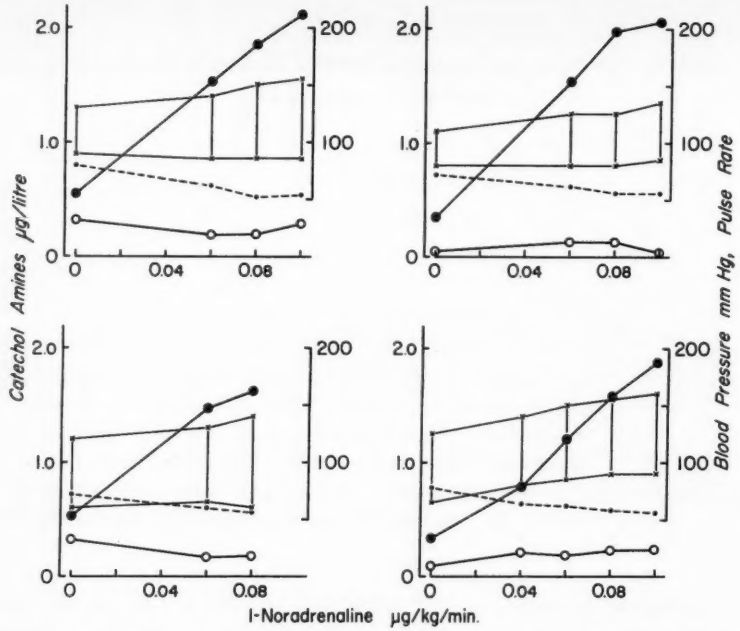


Figure 9. Comparison of separate infusions of noradrenaline in four subjects. Plasma concentrations in peripheral vein were measured for each infusion speed after 15 minutes had elapsed, *i.e.* at the steady state.

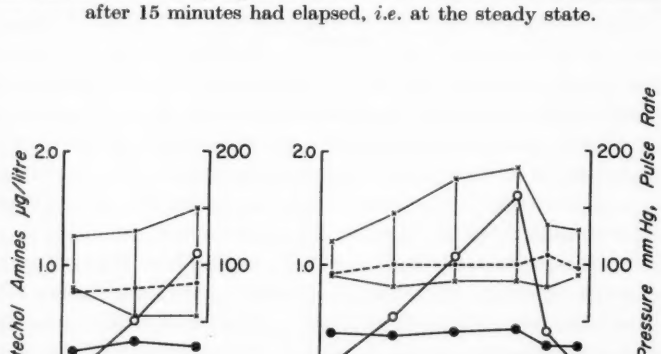


Figure 10. Comparison of separate infusions of adrenaline in two subjects. Plasma concentrations in peripheral vein were measured for each infusion speed after 15 minutes had elapsed.

●——● noradrenaline; ○——○ adrenaline; - - - - pulse; ×——× blood pressure

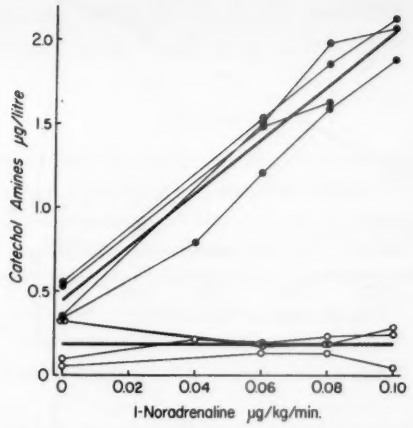


Figure 11. The steady state plasma concentrations of infused noradrenaline in four subjects related linearily to the infusion rate. Plasma concentrations in peripheral vein were measured for each infusion speed after 15 minutes had elapsed.
●——● noradrenaline; ○——○ adrenaline.

renaline are shown. Of particular interest was the finding that noradrenaline attained a higher concentration in plasma for comparable infusion speeds than did adrenaline.

The steady state data obtained during four noradrenaline infusions have been plotted in Figure 11. There was little variation from one individual to another in the steady state concentration of catechol amines; the data lay along a fairly well defined line. The highest plasma concentration of the infused catechol amines during these studies lay around 2.5 µg per litre for noradrenaline infusions and 2 µg per litre for adrenaline infusions. These values were four or ten times higher than the highest normal resting values observed, *viz.*, 0.64 µg per litre for noradrenaline and 0.22 µg per litre for adrenaline.

In a few studies blood specimens were taken at various time intervals after cessation of infusion, in order to determine the rate of decay of infused catechol amines in the plasma. Decay was rapid with a half-life of about 2½ minutes for noradrenaline. A more precise evaluation of this quantity is attempted in the Discussion section.

In three experiments intravenous infusions of noradrenaline were performed at successively higher rates, and concentrations of catechol amines in the peripheral and hepatic vein were observed. The changes in the concentration of infused catechol amines in the hepatic vein

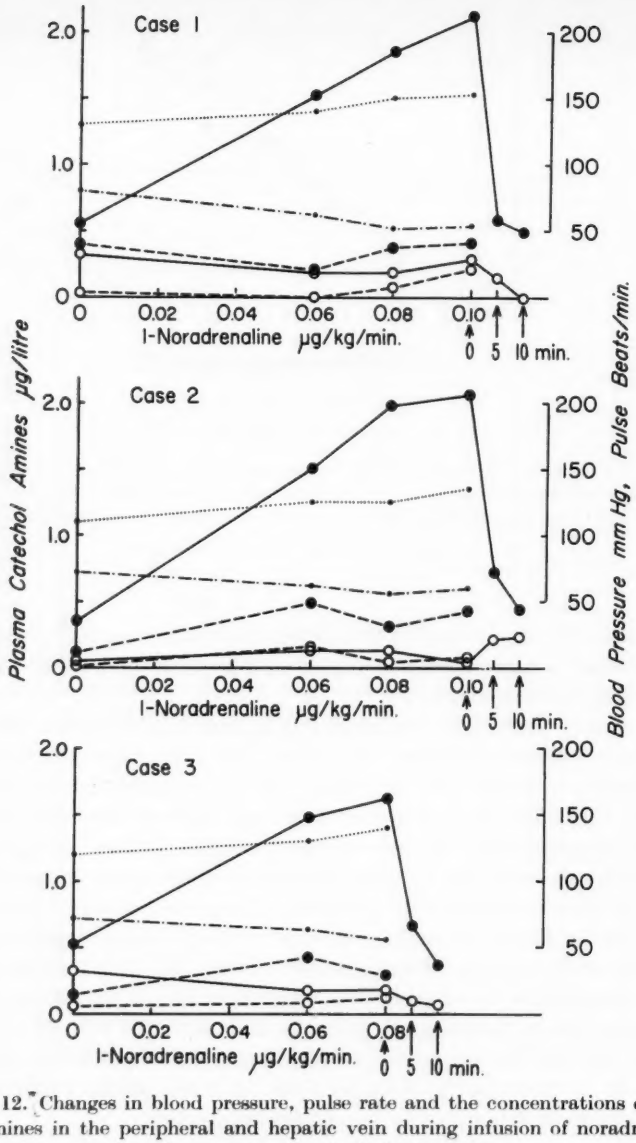


Figure 12. Changes in blood pressure, pulse rate and the concentrations of catechol amines in the peripheral and hepatic vein during infusion of noradrenaline performed at successively higher rates.

●——● noradrenaline in peripheral vein; ○——○ adrenaline in peripheral vein;
●- - -● noradrenaline in hepatic vein; ○- - -○ adrenaline in hepatic vein;
blood pressure; –·–·– pulse.

(It should be noted that the axis of abscissae is used also as time scale after the points where infusion ceased).

were small, indicating diffusion, metabolic transformation, or binding to tissues in the region of portal circulation. See Table 10 and Figure 12.

Discussion

During a constant rate infusion, the steady state represented a condition in which the rate of addition of catechol amine to plasma was equal to the summation of the rates of the various processes causing the disappearance of this catechol amine from the plasma (*viz.*, diffusion, excretion, metabolic transformation, changes in the circulating blood volume and so on). The data indicate lack of significant endogenous contributions of adrenaline and noradrenaline from the body stores and sites of synthesis such as the suprarenal glands and sympathetic nerves.

The relatively higher concentrations attained by noradrenaline than by adrenaline during infusion at equivalent rates indicated that adrenaline was removed from the circulation more quickly than noradrenaline. Adrenaline infusion is known to increase blood flow through the liver whereas noradrenaline infusion does not (Bearn, Billing and Sherlock, 1951). Since the liver has been demonstrated to be a major site for adrenaline and noradrenaline inactivation (Lund, 1951), it follows that increased liver blood flow associated with infusion of adrenaline may have resulted in lower steady state concentrations. However, further specific differences in the rates of removal of one or other of the two amines at other sites may be more important.

Estimation of resting secretion rates. Estimates of the average rates at which endogenous substances reach the circulating plasma under resting conditions were calculated from the linear relationship between infusion rates and steady state concentrations, and from the assumption that resting values were observed under steady state conditions. The calculations were made following those of Cohen, Holland, Sha and Goldenberg (1959). The values calculated by these authors are shown in parentheses below those obtained here.

Noradrenaline, 0.35 μg/litre

$$\times \frac{0.1\ \mu g/kg/min.}{2.0\ \mu g/litre} = \underset{(0.04)}{0.018\ \mu g/kg/min.}$$

Adrenaline, 0.06 μg/litre

$$\times \frac{0.21\ \mu g/kg/min.}{1.6\ \mu g/litre} = \underset{(0.01)}{0.008\ \mu g/kg/min.}$$

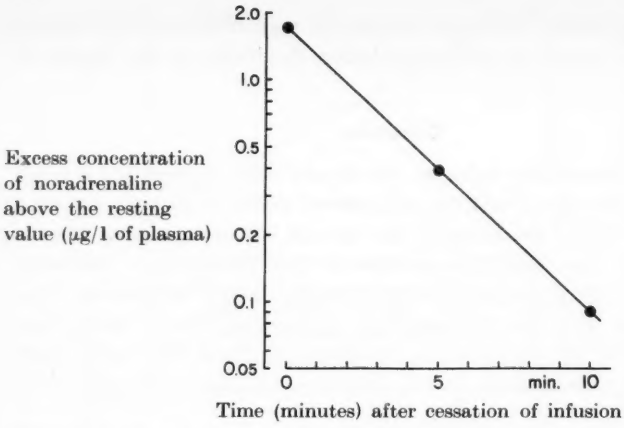


Figure 13. The rate of return of the concentration of noradrenaline in the peripheral venous plasma to the resting level after stopping the infusion of this substance (Case No. 2).

It should be noted that the major portion of endogenous noradrenaline is produced by sympathetic nerves (EULER, FRANKSSON and HELLSTRÖM, 1954) which do not release their secretion directly into the general circulation. Consequently only the portion which escapes local elimination, and which subsequently enters the circulatory system, is reflected in the plasma concentration. Therefore this average endogenous secretion rate calculated for noradrenaline should be distinguished from the average total endogenous secretion rate, of which the rate calculated above represents a lower limit. In comparison, the major portion of endogenous adrenaline enters the circulation directly *via* the suprarenal veins and hence the endogenous secretion rate for adrenaline may be calculated directly as shown above.

The half-life of noradrenaline in the blood. In Table 10 attention is drawn to the differences in the resting levels of adrenaline between case 2 on the one hand and cases 1 and 3 on the other. Case 2 had a resting level well within the normal range (see Table 7). While cases 1 and 3 had substantially raised levels (greater than four standard deviations above the mean value) indicating that they were nervous, and this is also reflected in the raised values of the "resting" levels of noradrenaline in these same two subjects. This fact makes calculation of the half-life of noradrenaline unreliable in cases 1 and 3. But case 2 not only had a normal resting level of adrenaline, but also the level of noradrenaline in this subject was exactly equal to the mean value (see Table 7). Therefore

Table 10. *Plasma concentrations (μg/litre) of adrenaline (A) and noradrenaline (NA) in peripheral and hepatic vein during intravenous infusion of l-noradrenaline.*

		Infusion rate μg/kg/min.	Antecubital vein A	Antecubital vein NA	Hepatic vein A	Hepatic vein NA	Pulse per min.	Blood pressure mm Hg
Case 1	Before infusion		0.32	0.55	0.04	0.40	80	130/90
Male		0.06	0.18	1.53	0.00	0.21	62	140/85
Age: 23	During infusion	0.08	0.19	1.85	0.07	0.37	52	150/85
Weight: 58 kg		0.10	0.29	2.12	0.22	0.41	54	155/85
	5′ after infusion		0.15	0.58	—	—	—	—
	10′ » »		—0.01	0.49	—	—	—	—
Case 2	Before infusion		0.05	0.35	0.01	0.12	72	110/80
Male		0.06	0.13	1.50	0.16	0.49	62	125/80
Age: 24	During infusion	0.08	0.13	1.98	0.05	0.31	56	125/80
Weight: 77 kg		0.10	0.05	2.06	0.08	0.43	56	135/85
	5′ after infusion		0.22	0.73	—	—	—	—
	10′ » »		0.23	0.44	—	—	—	—
Case 3	Before infusion		0.32	0.53	0.06	0.14	72	120/60
Male		0.06	0.17	1.48	0.08	0.43	60	130/65
Age: 27	During infusion	0.08	0.18	1.62	0.12	0.29	56	140/60
Weight: 75 kg								
	5′ after infusion		0.10	0.66	—	—	—	—
	10′ » »		0.07	0.37	—	—	—	—

using the data from this subject to calculate the rate of return of the noradrenaline concentration to the resting level, the results shown in Figure 13 were obtained. It may be seen that the disappearance of noradrenaline from the blood of case 2 was a simple first-order (logarithmic) process and that the half-life of the substance was 2.3 minutes (corresponding with a turnover time of $3\,^1/_3$ minutes). On comparing this value with the values calculated in the previous chapter, the result suggests that both hepatic and renal mechanisms of removal might be involved to some extent, although this is not by any means shown unambiguously. In fact the values found (Table 3 in the Appendix) for noradrenaline in either the left or the right renal vein, when accompanied by a low adrenaline value, were often close to the value in the antecubital vein; the question of the extent of participation by the kidney in the removal of noradrenaline must be left open. It can be stated more definitely that approximately 30 per cent of the blood volume is cleared of noradrenaline every minute, without prejudice to the question of the exact sites where this occurs. COHEN, HOLLAND, SHA and GOLDENBERG (1959) calculated a turnover time of 30 seconds for both adrenaline and

noradrenaline from the steady state concentrations at a known rate of infusion. Comparison of their values for the steady state concentrations with those obtained in the present work at similar rates of infusion shows that higher values were obtained here using the modified spectrophotofluorometric technique. Calculation of the turnover time in a similar way from the data of Figure 12 gives a value of about 1 minute. It is considered, however, that analysis of the data as shown in Figure 13 is likely to yield more reliable values as the measurements of the rate of removal are more direct, and evaluation of the rate is not dependent on the accuracy of measuring the absolute concentrations.

Chapter VI

Plasma Concentrations of Adrenaline and Noradrenaline during Muscular Work

An increase in suprarenal medullary secretion during muscular work has been suggested by studies on experimental animals (HARTMAN, WAITE and MCCORDOCK, 1922, on the denervated iris; CANNON, LINTON and LINTON, 1924, HOUSSAY and MOLINELLI, 1925, on the denervated heart). MOLINELLI (1926), however, measured the glycemic response in cross-circulation experiments and found no definite effect of muscular activity induced by electric stimulation. Estimating the active substances in suprarenal venous blood, WADA, SEO and ABE (1935) found unaltered medullary secretion in non-fatigued animals, while in fatigued animals the secretion, estimated as adrenaline, showed a marked increase. After muscular work the chromaffinity of the suprarenal medullary cells has been found to be reduced (SELYE, 1936), and the content of adrenaline in the suprarenal glands has been found to be decreased (HÖKFELT, 1951).

The observations by FREEMAN and ROSENBLUETH (1931) that muscular work was accompanied by a considerable fall in blood pressure in sympathectomized cats, implied an increased activity of the adrenergic nerves during muscular activity. LORD and HINTON (1945) and HOLMGREN (1955), similarly found a fall in blood pressure in man during muscular work after sympathectomy.

The vasodilatation which accompanies muscular activity is normally compensated for by the homeostatic mechanisms controlling blood pressure, as indicated by the failure of the pressure to be maintained during muscular work after exclusion of the pressor-regulating systems (EULER and LILJESTRAND, 1946). The compensatory vasoconstriction in other areas would also imply an increased activity of the adrenergic nerves (primarily of the vasomotor nerves which quantitatively constitute the most important part of the adrenergic system); the enhanced adrenergic nervous activity in all areas might then be followed by liberation of the transmitter substance, noradrenaline, into the circulating blood.

EULER and HELLNER (1952) were able to demonstrate an increased

excretion in man of adrenaline and noradrenaline in the urine during strenuous muscular work, while during slight or moderate work the increase was insignificant. There was no appreciable change in the relative proportions of adrenaline and noradrenaline as compared with the resting value. The excess excretion was thought to derive both from the suprarenal medulla and from the adrenergic nerves. In agreement with the results of EULER and HELLNER (1952), HOLMGREN (1955) similarly found a proportional increase in the urinary excretion of both adrenaline and noradrenaline during muscular work. No relationship could be established between, on the one hand, the rates of excretion of catechol amines and, on the other hand, the absolute and relative work intensity of the highest load, the arterial blood pressure during work, or the lactic acid concentration in the arterial blood during the prolonged heavy work.

It was of interest to see whether more detailed information could be obtained which would throw further light on the results of EULER and HELLNER (1952), and HOLMGREN (1955).

Material and Methods

This series consisted of 59 healthy subjects — 41 males aged from 17 to 38, and 18 females aged from 17 to 48 (medical students, physicians, nurses, civil servants and active athletes). The work test with successively increased loads (SJÖSTRAND, 1947) was performed on an electrically braked bicycle ergometer (HOLMGREN and MATTSSON, 1954).

All subjects were examined clinically with auscultation of the heart, electrocardiogram at rest and roentgenography of the heart and the lungs. The hemoglobin concentration in finger blood, the sedimentation rate and the urine analysis were normal. Next day the subjects returned to the laboratory and were first allowed to rest for about half an hour. Electrocardiogram electrodes were taped on the chest. After the introduction of an ordinary Cournand needle which was lodged in the antecubital vein, the blood samples were taken. The subjects were then placed on the bicycle ergometer. They were told to take the most convenient position which they could maintain during the work.

The subjects were divided into two groups according to sex, and each group was further divided into three according to their different working capacities. With males the work was started with a first load of 300 kgm/min. The work consisted of an uninterrupted series of loads, successively increasing by 300 kgm/min. every 6½ minutes. The first group, consisting of only 2 subjects, performed work with loads up to 600

kgm/min., the second group, comprising 21 subjects, with loads up to 900 kgm/min. and the third group, 18 subjects, with as much as 1200 kgm/min. With females the work test began with a load of 200 kgm/min. followed by uninterrupted series of loads, successively increasing by 200 kgm/min. every 6½ minutes. The first group, comprising 4 subjects, performed work with loads up to 400 kgm/min. In the second group, consisting of 10 subjects, the load was increased to 600 kgm/min. and in the third group, consisting of 4 subjects, to 800 kgm/min. The loads were increased until the subjects had reached a pulse rate of about 150—170 beats per minute. During work, repeated observations of pulse and respiratory rates, blood pressure and electrocardiogram were made. The pulse rate was counted and the blood pressure registered every two minutes during each load. The respiratory rate was counted every three minutes. The majority of subjects were in an approximately steady state (*i.e.* 10 beats/min. or less change of pulse rate from the second to the sixth minute of work) at the highest load. The blood samples were taken through the needle at the sixth minute of each load.

Results

The relative work intensity at the various loads is expressed as a pulse rate for the different groups in the Tables 11 and 13 for healthy men and women, respectively. The linear relationship between load and pulse rate is evident in all groups.

As to the males, the "600" group worked with a pulse rate of 161±11 beats/min. after 6 minutes at the highest load, the "900" group with 161±3.2 and the "1200" group with a pulse rate of 165±2.6 beats per minute after 6 minutes at the highest load. The systolic blood pressure response was closely correlated with the absolute load, every increase of the load being followed by an increase of the pressure. The mean values of the systolic pressure at rest for the "600", the "900" and the "1200" groups were practically identical; the pressure values at the highest loads were 188±22, 189±3.7 and 208±4.6 mm Hg in the different groups, respectively.

The mean concentrations of adrenaline and noradrenaline during rest did not differ significantly between different groups. During successively increasing uninterrupted series of loads, successively increased concentrations of noradrenaline were noted in all groups. See Table 12 and 14 and Figure 14.

In males, the mean concentrations of noradrenaline in peripheral venous plasma were, at the highest load, 0.78±0.05 μg, 1.20±0.07 μg

Table 11. *Pulse rate (per min.) and systolic blood pressure (mm Hg) in healthy men at rest and during muscular work with successively increased loads.*

Group		Resting		During work								5′ after work		10′ after work	
				300 kgm/min. 6′		600 kgm/min. 6′		900 kgm/min. 6′		1200 kgm/m. 6′					
		Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure
"600"	Mean	71	125	129	168	161	188	—	—	—	—	87	133	80	128
	S.E.	±11.5	±0.0	±16.0	±12.5	±11.0	±22.5	—	—	—	—	±4.5	±12.5	±7.0	±12.5
	n	2	2	2	2	2	2	—	—	—	—	2	2	2	2
"900"	Mean	62	125	100	149	128	171	161	189	—	—	80	131	76	127
	S.E.	±2.1	±1.9	±2.6	±3.0	±2.7	±2.9	±3.2	±3.7	—	—	±3.2	±2.6	±3.2	±2.8
	n	21	21	21	21	21	21	21	21	—	—	20	20	20	20
"1200"	Mean	58	126	90	151	111	169	138	191	165	208	76	131	74	124
	S.E.	±2.5	±2.0	±2.7	±4.1	±2.8	±3.8	±3.0	±4.4	±2.6	±4.6	±3.1	±2.2	±3.0	±1.7
	n	18	18	18	18	18	18	18	18	18	18	18	18	18	18

Table 12. *Plasma levels (μg/litre) of adrenaline (A) and noradrenaline (NA) in healthy men at rest and during muscular work with successively increased loads.*

Group		Resting		During work								5′ after work	
				300 kgm/min. 6′		600 kgm/min. 6′		900 kgm/min. 6′		1200 kgm/min. 6′			
		A	NA	A	NA	A	NA	A	NA	A	NA	A	NA
"600"	Mean	0.07	0.42	0.10	0.56	0.22	0.78	—	—	—	—	0.18	0.50
	S.E.	±0.03	±0.05	±0.00	±0.05	±0.03	±0.05	—	—	—	—	±0.07	±0.24
	n	2	2	2	2	2	2	—	—	—	—	2	2
"900"	Mean	0.08	0.31	0.11	0.44	0.18	0.72	0.27	1.20	—	—	0.23	0.34
	S.E.	±0.02	±0.02	±0.01	±0.02	±0.02	±0.03	±0.02	±0.07	—	—	±0.04	±0.05
	n	21	21	21	21	21	21	21	21	—	—	13	13
"1200"	Mean	0.06	0.30	0.12	0.43	0.18	0.62	0.25	0.95	0.34	1.67	—	—
	S.E.	±0.02	±0.01	±0.02	±0.02	±0.03	±0.03	±0.03	±0.06	±0.03	±0.09	—	—
	n	18	18	18	18	18	18	18	18	18	18		

Table 13. *Pulse rate (per min.) and systolic blood pressure (mm Hg) in healthy women at rest and during muscular work with successively increased loads.*

Group		Resting		During work								5′ after work		10′ after work	
				200 kgm/min. 6′		400 kgm/min. 6′		600 kgm/min. 6′		800 kgm/min. 6′					
		Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure
"400"	Mean	78	119	127	146	164	158	—	—	—	—	92	120	89	122
	S.E.	±9.0	±5.5	±4.4	±10.5	±3.1	±7.8	—	—	—	—	±9.1	±4.6	±10.7	±4.4
	n	4	4	4	4	4	4	—	—	—	—	4	4	3	3
"600"	Mean	71	118	111	144	132	155	162	167	—	—	84	122	81	116
	S.E.	±3.0	±3.1	±3.0	±3.7	±3.8	±2.6	±5.3	±3.2	—	—	±4.8	±2.1	±4.9	±3.5
	n	10	10	10	10	10	10	10	10	—	—	10	10	10	10
"800"	Mean	72	121	105	144	120	153	147	170	173	183	88	125	87	120
	S.E.	±4.9	±6.6	±6.8	±12.1	±4.2	±7.8	±7.7	±4.1	±4.5	±6.3	±2.6	±6.5	±3.2	±10.4
	n	4	4	4	4	4	4	4	4	4	4	4	4	3	3

Table 14. *Plasma levels (μg/litre) of adrenaline (A) and noradrenaline (NA) in healthy women at rest and during muscular work with successively increased loads.*

Group		Resting		During work								5′ after work		10′ after work	
				200 kgm/min. 6′		400 kgm/min. 6′		600 kgm/min. 6′		800 kgm/min. 6′					
		A	NA	A	NA	A	NA	A	NA	A	NA	A	NA	A	NA
"400"	Mean	0.06	0.37	0.14	0.50	0.23	0.71	—	—	—	—	0.14	0.43	—	—
	S.E.	±0.02	±0.05	±0.01	±0.07	±0.04	±0.08	—	—	—	—	±0.00	±0.15	—	—
n	4	4	4	4	4	4	4	—	—	—	—	2	2	—	—
"600"	Mean	0.05	0.35	0.09	0.44	0.15	0.58	0.18	0.91	—	—	0.05	0.30	0.04	0.31
	S.E.	±0.01	±0.04	±0.02	±0.05	±0.03	±0.06	±0.03	±0.10	—	—	±0.04	±0.04	±0.02	±0.03
	n	10	10	10	10	10	10	10	10	—	—	4	4	3	3
"800"	Mean	0.07	0.32	0.08	0.47	0.19	0.59	0.20	0.88	0.30	1.30	—	—	—	
	S.E.	±0.01	±0.02	±0.02	±0.04	±0.05	±0.09	±0.03	±0.07	±0.03	±0.05	—	—	—	
	n	4	4	4	4	4	4	4	4	4	4	—	—	—	

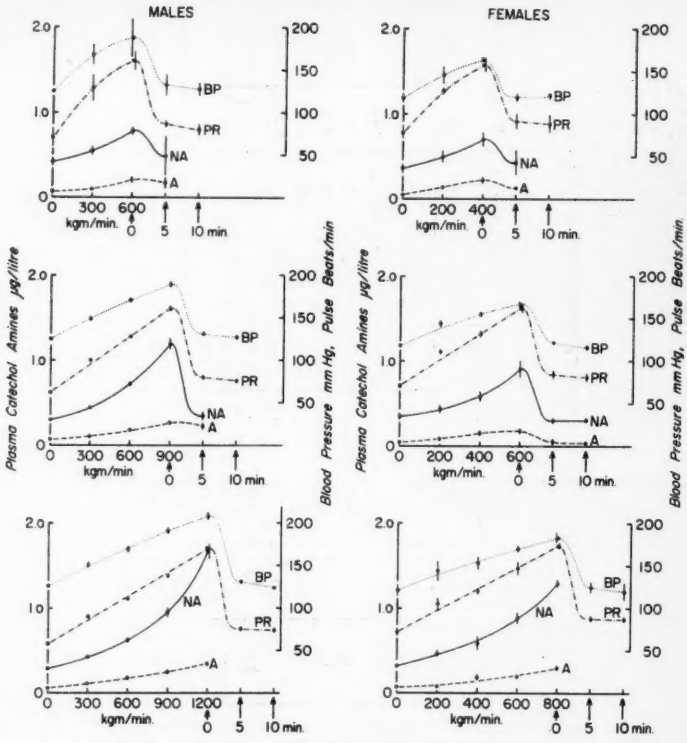
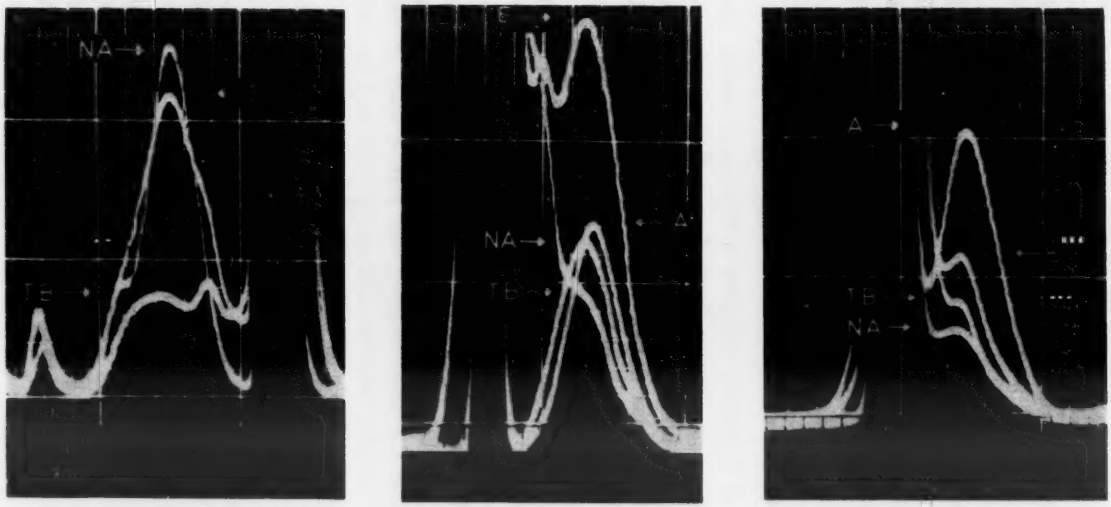


Figure 14. Changes in blood pressure (BP), pulse rate (PR), and the concentrations of adrenaline (A) and noradrenaline (NA) during exercise in six groups of healthy subjects. (It should be noted that the axis of abscissae is used also as a time scale after the points where exercise ceased.)

and 1.67 ± 0.09µg per litre of plasma, respectively, in the foregoing groups. The mean concentrations of adrenaline at the highest loads in the "600", "900" and "1200" groups were 0.22 ± 0.03 µg, 0.27 ± 0.02 µg and 0.34 ± 0.03 µg per litre of plasma, respectively.

With the females, the "400 "group worked with a pulse rate of 164 ± 3.1 beats/min. after 6 minutes at the highest load, the "600" group with 162 ± 5.3 and the "800" group with a pulse rate of 170 ± 4.1 beats/min. at the highest load. The systolic pressure at rest had an average value of 119 ± 5.5 mm Hg for the "400" group, 118 ± 3.1 mm Hg for the "600" group and 121 ± 6.6 mm Hg for the "600" group and 121 ± 6.6 mm Hg for the "800" group, the values being 158 ± 7.8, 167 ± 3.2 and 183 ± 6.3 mm



I II III

Figure 15. Spectra of an eluate from a blood sample drawn from the antecubital vein during work. The blood sample was taken at the highest load (1200 kgm/min. = maximal physical working capacity for this individual Case No. 25, Table 5 in the Appendix).

I Activation spectrum. Fluorescence wave-length 540 mμ.
II Fluorescence spectrum. Activating wave-length 410 mμ.
III Fluorescence spectrum. Activating wave-length 455 mμ.

A Adrenaline standard; NA Noradrenaline standard; E Eluate; TB Faded sample blank.

The spectra of the eluate come close to those obtained from noradrenaline.

Hg at the highest loads in the different groups, respectively. The mean concentrations of noradrenaline in peripheral venous plasma were, at the highest load, 0.71 ± 0.08 µg, 0.91 ± 0.10 µg and 1.30 ± 0.05 µg per litre of plasma, respectively, in the mentioned groups. The mean concentration of adrenaline at the highest load in the "400", "600" and "800" groups were 0.23 ± 0.04 µg, 0.18 ± 0.03 µg and 0.30 ± 0.03 µg per litre of plasma, respectively.

Five minutes after work the concentrations of noradrenaline had fallen almost to the values at rest. Further details are supplied in Tables 4, 5, 6 and 7 in the Appendix. The spectra of an eluate from a blood sample drawn from the antecubital vein during work are shown in Figure 15.

The Source of Noradrenaline

Methods. In a series of 5 healthy subjects a Cournand needle was lodged in the antecubital vein and a polyethylene catheter was percutaneously introduced into the femoral vein. After the blood samples had been taken from these veins during rest the subjects performed work with a load of 900 kgm/min. Blood samples were taken at the sixth minute of work for analysis.

In a second series comprising three healthy subjects (medical students) the tip of a Cournand catheter was inserted about 1 to 2 cm into the left renal vein under fluoroscopic control. The subjects were lying in the supine position during the whole procedure, including the work test on the bicycle ergometer. Two healthy subjects performed work with a load of 600 kgm/min. and the third healthy one with a load of 900 kgm/min. Double blood samples from the antecubital vein and the left renal vein were taken simultaneously after 6 minutes' work.

Results

Table 15 shows the plasma concentrations of adrenaline and noradrenaline in the antecubital and femoral veins at rest and during work on the bicycle e gometer. The mean concentrations obtained from the antecubital and the femoral veins at rest were very similar ($p > 0.25$ and $p > 0.50$, respectively). The increased mean concentrations of adrenaline and noradrenaline obtained from these veins during work showed no significant difference when compared to each other ($p > 0.50$ and $p > 0.10$, respectively).

Plasma levels of adrenaline and noradrenaline in peripheral venous blood and in the left renal vein before and during muscular work are

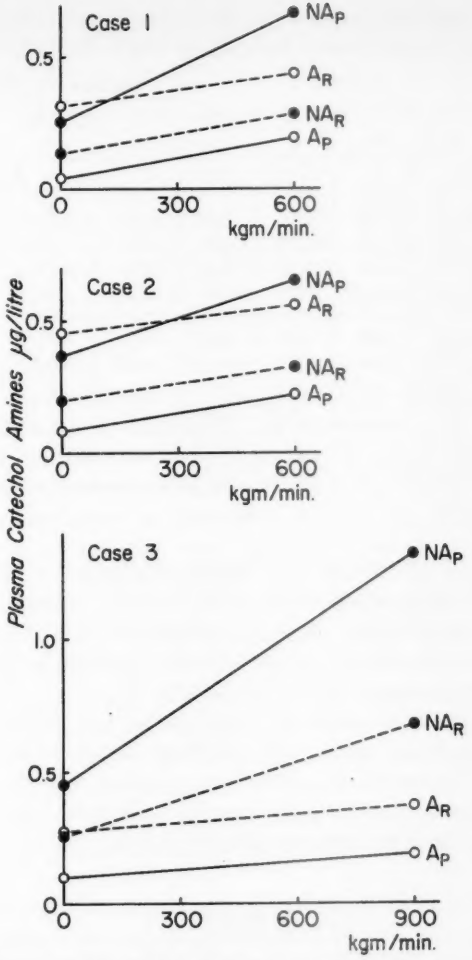


Figure 16. Plasma levels of adrenaline and noradrenaline in peripheral venous blood and in the left renal vein before and during muscular work.
●——● noradrenaline, peripheral vein; ○——○ adrenaline, peripheral vein;
●- - -● noradrenaline, left renal vein; ○- - -○ adrenaline, left renal vein.

presented in Table 16 and Figure 16. The values obtained from the antecubital vein at rest show good agreement with the values of the normal material, consisting of 125 subjects (Table 7). The concentrations of adrenaline and noradrenaline obtained in the left renal vein showed a

Table 15. *Plasma concentrations (μg/litre) of adrenaline (A) and noradrenaline (NA) in antecubital and femoral vein at rest and during work on the bicycle ergometer.*

Cace No.	Age	Sex	Resting				During work, 900 kgm/min. 6′			
			Antecubital vein		Femoral vein		Antecubital vein		Femoral vein	
			A	NA	A	NA	A	NA	A	NA
1	36	M	0.10	0.43	0.05	0.39	0.11	0.90	0.08	0.95
2	32	M	–0.04	0.32	–0.04	0.34	0.19	0.92	0.15	1.05
3	23	M	–0.04	0.34	–0.04	0.36	0.13	1.10	0.16	1.10
4	21	M	0.04	0.34	0.06	0.42	0.28	0.87	0.32	1.00
5	37	M	0.33	0.54	0.31	0.50	0.36	1.30	0.42	1.27
Mean			0.08	0.39	0.07	0.40	0.22	1.02	0.23	1.07
S.E.			±0.07	±0.04	±0.06	±0.03	±0.05	±0.08	±0.06	±0.05
n			5	5	5	5	5	5	5	5
Diff.*					−0.01	±0.01			+0.01	+0.06
S.E.					±0.01	±0.02			±0.02	±0.03
					p>0.25	p>0.50			p>0.50	p>0.10

preponderance of adrenaline, the values ranging from 0.27 to 0.45 μg per litre. The noradrenaline values were lower in comparison with those of adrenaline, ranging from 0.13 to 0.26 μg per litre of plasma. These values conform well with the concentrations obtained from the left renal vein by catheterization, presented in Chapter IV.

The plasma levels of noradrenaline obtained from the peripheral vein, at rest as well as during work, exceeded those obtained from the left renal vein. The concentrations of adrenaline increased slightly. The concentrations of adrenaline obtained from the left renal vein in each case exceeded those obtained from the peripheral vein.

Discussion

It may be seen that the same work loads, supported for the same length of time, elicited an approximately similar increase in level of noradrenaline in all six groups. There appears, therefore, to be no effect of training on the levels observed, the effect of training being rather on the maximum working capacity.

The data of Figure 14 show that in all groups there was a small increase in the level of adrenaline. In marked contrast to this, the levels of noradrenaline increased rapidly and exponentially with the load; the rapid increase in concentration as the load was increased provides support for the 'overflow' hypothesis of CANNON and ROSENBLUETH

Table 16. *Plasma levels (μg/litre) of adrenaline (A) and noradrenaline (NA) in peripheral venous blood and in left renal vein during muscular work.*

Cases			Resting				Loads in kgm/min		During work			
			Antecubital vein		Left renal vein				Antecubital vein		Left renal vein	
No.	Age	Sex	A	NA	A	NA			A	NA	A	NA
1	27	M	0.04	0.25	0.31	0.13	600	6′	0.19	0.67	0.43	0.28
2	27	M	0.08	0.37	0.45	0.20	600	6′	0.22	0.65	0.56	0.33
3	22	M	0.10	0.45	0.27	0.26	900	6′	0.19	1.32	0.37	0.08

(1937, 1949; ROSENBLUETH, 1950) in which it was proposed that effector substances released at adrenergic nerve-endings may, under certain circumstances, escape into the blood stream. It is not clear why the level of noradrenaline increases so rapidly at the higher loads, but the form of the curve is suggestive of saturation of enzyme systems destroying noradrenaline and situated very near the sites of production in the peripheral tissues. Infusion studies (Chapter V) have shown that increase in concentration of noradrenaline proceeds linearly with the rate of infusion, up to blood levels substantially higher than those observed in the studies during exercise. By analogy it might be expected that elevation of noradrenaline concentration, resulting from increased rates of release in the peripheral tissues during exercise, would also proceed linearly with the intensity of the stimulus. It is, however, not impossible that, while being unimportant in the removal of noradrenaline from the blood, localized enzymic activity could be responsible for destroying noradrenaline produced locally in the peripheral tissues before it reaches the blood.

The experiments involving catheterization of the left renal vein indicate that, there is no major contribution by the suprarenal glands to the increased amounts of this substance secreted during exercise.

There were no marked differences (Table 15) in the concentrations of noradrenaline in the femoral and the antecubital venous blood. This shows that enhanced peripheral release of noradrenaline during exercise is, as would be expected with a substance secreted at sympathetic nerve-endings, generalized throughout the body rather than a localized phenomenon confined to the venous blood returning from regions of the body where the work was being done.

Chapter VII

The Effect of Body Posture on the Concentration of Adrenaline and Noradrenaline in Plasma

The hemodynamic adjustment to an abrupt change in body position from the horizontal to the vertical has been extensively studied (for literature, see HICKLER, WELLS, TYLER and HAMLIN, 1959). Assumption of the upright position results in pooling of blood in the splanchnic area and in the lower extremities, consequently, there is a reduction in the circulating blood volume, decreased venous return and diminished cardiac output. The orthostatic fall in blood pressure brings into play the homeostatic reflex mechanisms; this results in a general stimulation of the vasomotor nerves, the process being mediated by the baroceptive zones in the aorta and the carotid sinus.

The combined effect of the various homeostatic processes in operation during the change from the recumbent to the erect posture is reflected in the following physical findings in healthy subjects or in subjects with orthostatic hypotension.

In *healthy* subjects there is often a slight rise in the pulse rate and diastolic blood pressure with no change in systolic pressure (ASMUSSEN, CHRISTENSEN and NIELSEN, 1939).

Of the two forms of *orthostatic hypotension*, one, *arterial orthostatic anemia* (BJURE and LAURELL, 1927) or hypotonic regulatory disturbance (SCHELLONG, 1931), common in asthenic persons, is characterized by a markedly accelerated pulse rate and fall of the systolic blood pressure with or without rise of the diastolic pressure. In the second form, the *postural hypotension* or hypodynamic regulatory disturbance, the systolic and diastolic pressures fall upon assuming the upright position; the pulse rate, however, is not affected (BRADBURY and EGGLESTON, 1925).

The increased vasomotor activity during standing is accompanied, as might be expected, by an increased endogenous production of noradrenaline and as a result of this an increased urinary output of this substance (EULER, 1954, EULER, LUFT and SUNDIN, 1955, SUNDIN, 1956, 1958). Correspondingly, in two cases of postural hypotension, LUFT and EULER (1953) observed a decreased excretion of noradrenaline and adrenaline.

The increased urinary output of noradrenaline is assumed to be due partly to an activation of the vasomotor system, and partly to an increase in the secretion of the suprarenal medulla, since it is known that when the baroceptor impulses coming from the carotid sinus are blocked, this is followed by an increased output of noradrenaline from the suprarenal medulla in the cat (KAINDL and EULER, 1951).

In order to obtain information regarding the temporal relationship between the orthostatic stress and release of catechol amines in the circulation, serial measurements of changes in the plasma concentration of the catechol amines were carried out.

Method and Material

All subjects were ambulant and were studied in the morning. The subject was placed in the horizontal, supine position on a tilting table with one pillow under the head for a period of 30 minutes prior to head-up tilt. Electrocardiographic tracings were recorded during the control period and the tilted position. During the tilting period every 30—60 seconds serial blood pressures were recorded from the left arm by auscultation with a standard aneroid blood pressure cuff. A Cournand needle was lodged in the right antecubital vein. Both the subject's arms remained at rest at his sides throughout the study. Venous blood samples were collected during the control period and two, five or ten minutes after tilting upright. The blood samples were collected directly in the silicone-coated centrifuge tubes and were chilled in ice-water until the complete set for the particular experiment had been collected. The time of tilting varied between five and ten minutes depending on the condition of the subject.

The present series comprised 17 healthy subjects whose age ranged between 19 and 65 years (Table 8, I, in the Appendix) and 23 subjects with a history of syncopal spells with ages ranging from 17 to 54 years. Fourteen subjects of the last group (Table 8, II, a, in the Appendix) were studied quite as long as the healthy subjects during the tilting period (*i.e.* for ten minutes), while the time of tilting for 7 subjects (Table 8, II, b, in the Appendix) was only five minutes, the test being interrupted because of threatening syncope. Syncope occurred in two subjects with a history of syncopal spells after four and eight minutes of tilting, respectively. The data relating to these subjects are presented separately, and are not included in the 23 subjects mentioned above. One subject with typical postural hypotension was also studied. The history of this patient may be summarized briefly as follows:

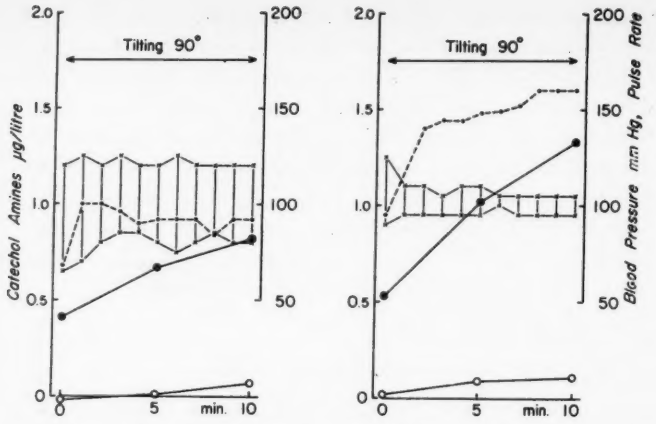


Figure 17. The catechol amine response during tilting for 10 minutes in a normal subject (Case No. 17, Table 8, I), to the left, and in a subject with arterial orthostatic anemia (Case No. 31, Table 8, II a), to the right.

●——● noradrenaline; ○——○ adrenaline; - - - - pulse; ×——× blood pressure.

A 70-year old man. The first symptom of his illness appeared 1½ years ago and consisted of vertigo, which made him almost completely incapacitated during the last months. About half a year later headache, localized to the occipital region, and difficulties in deglutition appeared. On hospitalization bilateral pseudobulbar palsy was diagnosed. Apart from the neurological signs the physiological examination was normal. Blood pressure varied between 130/70 and 150/100 mm Hg at rest. When standing up an immediate fall of the systolic pressure to 70—60 mm Hg was observed while the diastolic pressure fell to 50 mm Hg. The pulse rate remained unchanged. The concentration of noradrenaline and adrenaline in the peripheral venous plasma increased only slightly. The urinary excretion of catechol amines amounted to 36 µg per 24 hours. The patient died in pneumonia. Autopsy revealed an aneurysm (of the size of a walnut) in connection with left vertebral artery and there was compression of the medulla oblongata and pons.

Results

Healthy subjects

This series comprised 17 healthy subjects who were tilted to 90° from the horizontal for 10 minutes.

Hemodynamic response. The systolic blood pressure during recumbency before tilting, calculated as the average of the mean values for each individual was 119±3.1 mm Hg and 116±2.8 mm Hg during head-up tilt (variation is expressed as the standard error of the mean). The

diastolic blood pressure during the tilting period increased in comparison with the resting values; the mean value calculated from the total of each individual mean was 71±2.2 mm Hg before tilting and 83±2.3 during tilting. The mean pulse rate in recumbency was 77±2.7 beats per minute, and the corresponding value when the subjects were tilted was 97±2.5 (Table 8, I, in the Appendix). The difference was significant ($p<0.001$).

Catechol amine response. The mean concentrations of adrenaline and noradrenaline in recumbency were 0.04±0.01 μg and 0.36±0.02 μg per litre of plasma, respectively. After ten minutes of the head-up tilt the corresponding values were 0.11±0.02 μg and 0.62±0.03 μg per litre of plasma, respectively. The mean increase of adrenaline was 0.07 μg per litre of plasma, while the increase for noradrenaline was 0.26 μg per litre of plasma. The difference between the values in recumbence and the values during tilting was significant in respect of both adrenaline ($p<0.005$) and noradrenaline ($p<0.001$) concentrations.

In ten subjects of this series the blood samples were taken after only two minutes of tilting. The mean concentrations of adrenaline and noradrenaline at this time did not differ significantly from the resting values. In the remaining 7 subjects of the series the blood samples were taken after five minutes of tilting, the mean concentrations of noradrenaline showing an increase from 0.40 μg to 0.60 μg per litre plasma which was statistically significant ($p<0.005$), while the slight increase for adrenaline was not significant.

Arterial orthostatic anemia

This series comprised one group of 14 subjects enduring a tilt of 90° for a period of ten minutes and a second group of 7 subjects with threatening collapse after five minutes of tilting.

Hemodynamic response. In the first group (14 subjects) the mean systolic blood pressure during recumbency was 118±3.3 mm Hg and 103±4.9 mm Hg during tilting for ten minutes. The fall of systolic blood pressure was statistically significant ($p<0.001$). The mean value of diastolic blood pressure was 71±3.4 mm Hg before tilting and 82±3.3 mm Hg during tilting, the increase was significant ($p<0.005$). The mean pulse rate in recumbency was 77 beats per minute, the corresponding value when the subjects were tilted being 118. The mean increase calculated from the total of each individual's change in pulse rate was 41 beats per minute and was significant ($p<0.001$).

In the second group (7 subjects) the head-up tilt of 90° was interrupted

after five minutes because of premonitory symptoms and signs of threatening collapse. The subjects experienced epigastric discomfort and there was associated yawning and sighing, leading to hyperventilation and accompanied by sweating, pallor, pupillary dilatation and blurring of vision.

The mean systolic blood pressure during recumbency before tilting was 114 ± 4.6 mm Hg. During the head-up tilt for five minutes only one subject had a systolic blood pressure above 110 mm Hg. Two subjects had a systolic pressure below 100 mm Hg and in 4 subjects it was not measurable. The diastolic pressure increased in cases where the blood pressure was measurable. The mean pulse rate in recumbency was 73 ± 4.3 beats per minute and the corresponding value after 5 minutes of tilting was 104 ± 8.2.

Catechol amine response. In the first group (14 subjects) the mean concentrations of adrenaline and noradrenaline, in recumbency, were 0.05 ± 0.02 μg and 0.39 ± 0.02 μg per litre of plasma, respectively. The difference between recumbency values and tilting values was significant for noradrenaline ($p < 0.001$) but not for adrenaline. In 4 subjects of this group blood samples were taken after only two minutes of tilting. The mean concentrations of adrenaline and noradrenaline did not then differ significantly from the resting values. In the remaining 10 subjects the concentrations of noradrenaline measured after five minutes of tilting increased from 0.40 to 0.64 μg per litre of plasma. The difference was statistically significant. After 10 minutes head-up tilt the mean concentration of noradrenaline had increased to 0.82 μg/litre, which in comparison with the corresponding mean value of healthy subjects was significantly higher ($p < 0.01$) while there was no statistical difference in increase regarding the adrenaline value ($p > 0.5$) in these two groups. The catechol amine response in plasma in a normal subject and in a subject with arterial orthostatic anemia is shown in Figure 17.

In the group of 7 subjects where the test was interrupted after five minutes, the mean concentrations of adrenaline and noradrenaline before tilting were 0.05 ± 0.01 μg and 0.37 ± 0.04 μg per litre of plasma, the corresponding values after five minutes of tilting being 0.11 ± 0.3 μg and 0.83 ± 0.06 μg per litre, respectively. The mean concentration of noradrenaline was still increased five minutes after the subjects had assumed the supine position after tilting test.

In 2 subjects with arterial orthostatic anemia *syncope* occurred after four and eight minutes of tilting, respectively. The syncope in these 2 subjects was of the vasodepressor variety, with the usual prodromes of pallor, sweating, restlessness and sighing respiration. The vagotonic

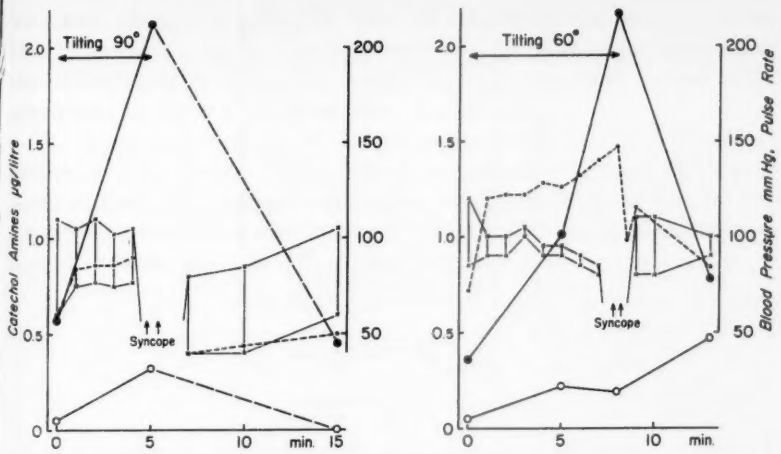


Figure 18. The catechol amine response during tilting in two subjects with syncope.

●——● noradrenaline; ○——○ adrenaline; - - - - pulse; ×——× blood pressure.

reaction was superimposed on a normal hemodynamic response to the orthostatic stress and was clearly substantiated by decreased pulse rate, occurring just before or at the time when the blood pressure suddenly fell to values not obtainable by auscultation. Syncope could not be related to deficiency in the catechol amine response, as the catechol amines rose to a significant peak just prior to the faint. Examples are shown in Figure 18. The figure shows the steep increase of the noradrenaline concentration following tilting, the maximum rise of the noradrenaline concentration occurring at the time of syncope, while a fall in pulse rate preceded the faint. The highest values for these 2 patients were above 2 µg of noradrenaline per litre of plasma.

Postural hypotension

The subject with postural hypotension had a prompt and progressive fall in systolic and diastolic blood pressure during head-up tilt of 65°. There was an insignificant rise in pulse rate despite marked hypotension. An insignificant change in adrenaline and noradrenaline concentration was accompanied by a significant fall in both the systolic and diastolic pressures. The results are shown graphically in Figure 19.

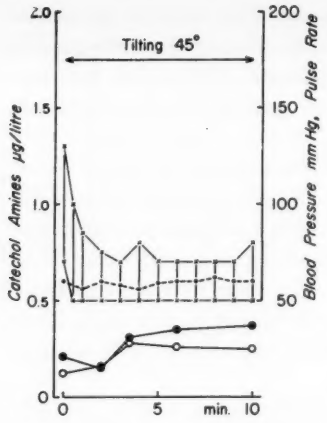


Figure 19. The catechol amine response during tilting in the subject with postural hypotension.

●——● noradrenaline; ○——○ adrenaline; - - - - pulse; ×——× blood pressure.

Discussion

A change from the recumbent to the upright position is normally accompanied by constriction both of the venous system in the lower part of the body and of the arteries (Asmussen, 1943).

Interferences with the regulatory reactions in orthostatic hypotension are reflected in more than one way. *Arterial orthostatic anemia* consists of an excessive pooling of blood in the veins below the heart due to lack of venous constriction (Weiss, 1935, Weiss, Wilkins and Haynes, 1937) and/or to insufficiency of the venous valves, while the reflex mechanism of arterial constriction remains more or less intact. The other type of orthostatic hypotension, designated as *postural hypotension*, shows a deficiency in compensatory arterial constriction (Nylin and Levander, 1948).

An increase in the concentration of noradrenaline in peripheral venous plasma was demonstrated in normal subjects, in all of whom the hemodynamic response to tilting upright was found to be normal. In the subjects with *arterial orthostatic anemia*, the increase of noradrenaline was more pronounced in comparison with the healthy group. Two subjects (see Figure 18) experiencing vasodepressor syncope superimposed on a hemodynamic response showed the highest concentrations of noradrenaline following orthostatic stress.

Acute changes in the plasma concentration of noradrenaline correlated

well with changes in arteriolar tone, as judged by maintenance of the level of diastolic pressure in spite of the tilt stress. Data presented support the concept that noradrenaline release by the sympathetic nerves is the important factor in the homeostatic mechanism.

The basic involvement of a sympathicotonic, *i.e.* neurosecretory, deficiency was obvious in the case of postural hypotension. It may be assumed that some failure in the reflex arc which is responsible for the liberation of catechol amines accounts for the failure to adjust the circulation to postural changes.

Chapter VIII

Plasma Catechol Amine Determinations in the Localization of Phaeochromocytoma

The estimation of catechol amines in urine may be of value in predicting the location of the tumour in the body preoperatively, as the differential assay of adrenaline and noradrenaline in the urine may suggest a suprarenal or an extra-suprarenal tumour site. In the collected series of 79 cases of phaeochromocytoma, CROUT and SJOERDSMA (1960) found that 78 per cent of all patients had tumors in the suprarenal region, and 95 per cent had tumours localized in the abdomen, the findings being comparable with those reported previously by GRAHAM (1951) and EULER and STRÖM (1957). If the urine contains increased amounts of adrenaline as well as noradrenaline the tumour is almost always in one of the suprarenal areas or more seldom in the organs of Zuckerkandl. If the increased excretion of catechol amines in the urine is limited to noradrenaline alone the tumour may still be expected to lie in or near one of the suprarenals in 2/3 of the cases in the remaining 1/3, however, all possible sites must be considered (CROUT and SJOERDSMA, 1960).

Measurement of the concentrations of catechol amines at different levels of the venae cavae can be of use both in diagnosing doubtful cases of phaeochromocytoma and as an aid to the surgeon by localization of the tumour (EULER and STRÖM, 1957, EULER, GEMZELL, STRÖM and WESTMAN, 1955, SCHAEPDRYVER, 1959, CROUT and SJOERDSMA, 1960).

In the following four cases, in which catheterization was employed, the method was found valuable. In one case where the resting concentrations of catechol amines were found to be normal, the procedure was successful when carried out in conjunction with a positive histamine test.

Case Reports and Catheterization Studies

Descriptions of each of the four cases in which catheterization of the vena cava was performed are presented individually, and the results of the 5 catheterizations are illustrated by figures for each separate case, illustrating the value of this technique.

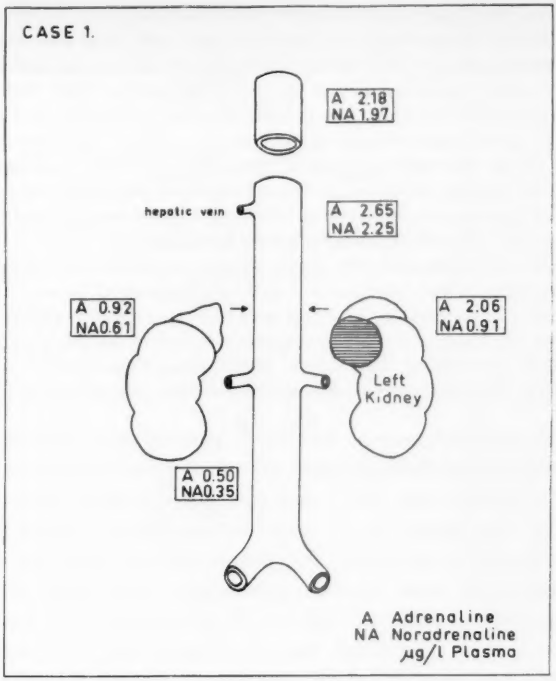


Figure 20.

Case 1. S.Å. A 34-year-old male who for three years had experienced recurrent nocturnal episodes of palpitation, perspiration, headache, apprehension and anxiety. These episodes lasted for about 10 minutes. In addition he had slight dyspnea on exertion. The patient was hospitalized in 1957 because of duodenal ulcer. The blood pressure was found to be normal, 130/70 mm Hg. A histamine test for the stimulation of gastric secretion initiated an episode of the above-mentioned character. On June 26, 1958 the patient got a trauma to the left flank. On hospitalization (the County Hospital of Sundsvall) a blood pressure of 220/80 mm Hg was noted. Considerable tenderness in the upper abdomen and the left flank was noted. The pain remained for more than a week, during which time the blood pressure varied between 150/80 and 235/135 mm Hg. Pulse rate remained about 100 per minute. A pronounced orthostatic reaction was noted with the blood pressure 235/135 in a reclining and 120/70 mm Hg in a sitting position. The Regitine test was positive twice. The second week after the trauma the patient felt much less discomfort and the blood pressure as well as the pulse rate showed decreasing values. After another week the blood pressure was normal. The daily urinary excretion of adrenaline and noradrenaline appears from Table 17.

An intravenous urogram showed that the left kidney was dislocated laterally

downwards, and the upper renal pole could not be delimited. On retroperitoneal gas insufflation no air concentration around the upper left renal pole was obtained. Since there was a suspicion of phaeochromocytoma in the left suprarenal gland, an exploratory incision was performed at the end of September, 1958. The incision for approach to the suprarenal gland was parallel with the twelfth rib. At the site of the left suprarenal gland, only sclerotic fatty connective tissue with necroses and hemorrhage was found. The right suprarenal gland was explored in the same way and was found to be normal. Positive histamine tests were performed twice in October and the blood pressure amounted from 130/70 to 170/70 mm Hg and from 120/80 to 230/90 mm Hg, respectively. Cold pressure test negative.

The patient was postoperatively asymptomatic except for occasional headache and excessive perspiration. Because of continued suspicion of phaeochromocytoma he was admitted to the General Hospital in Malmö for further evaluation.

On physical examination the patient appeared healthy. The blood pressure varied between 120/55 and 140/80 mm Hg. A plain roentgenogram showed kidneys of normal size and form and selective aortography showed nothing out of the ordinary.

The major problem was to localize a tumour in a patient, who had already undergone abdominal explorations for phaeochromocytoma. Vena cava catheterization was performed through the right antecubital vein and samples were drawn at six levels within the two venae cavae. The values corresponded well with the concentrations obtained in healthy subjects. Renewed vena caval catheterization was performed through the right saphenous vein and the tip of the catheter was lodged in the superior vena cava. The blood samples drawn were obtained after the intravenous injection of 75 μg of histamine (in terms of the base), which caused a great rise of blood pressure. The blood pressure returned to control levels about 8 minutes after the injection. The samples were drawn at four levels within the venae cavae. The sample from the superior vena cava was drawn two minutes and that from the upper part of the inferior vena cava three minutes after the injection of histamine. Four minutes after the injection of histamine a third set of samples was drawn at the level of the suprarenal area. One of the blood samples was taken with the tip of the catheter against the right lateral wall of the vessel and the other, 30 seconds later, with the tip against the left. Five and a half minutes after injection the sample from the lower part of the vena cava was taken. The catechol amine content of all the plasma samples was increased, and the highest value was found in the sample drawn from the upper part of the inferior vena cava. Of the two samples drawn from the level of the suprarenal area, the one that had been taken with the tip of the catheter to the left showed the highest value, Figure 20. The data were interpreted as demonstrating a tumour of the upper abdomen. The patient was subsequently explored through the longitudinal upper abdominal incision, and a phaeochromocytoma was found, connected

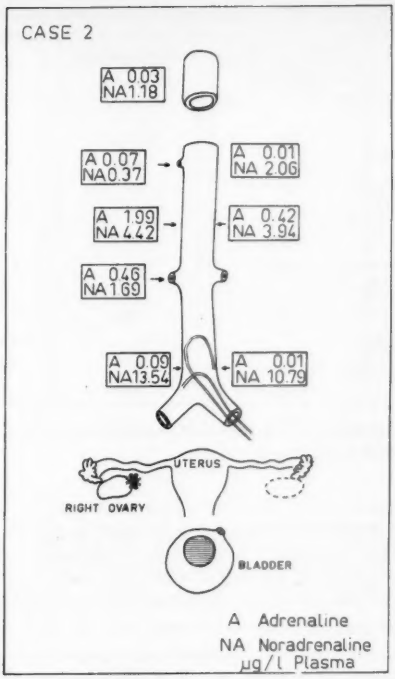


Figure 21.

with the left suprarenal gland. The excised tumour weighed 34 g and contained 7.25 mg adrenaline and 3.45 mg noradrenaline per gram tissue, adrenaline thus constituting no less than 66 per cent of the total catechol amines present.

In this case the blood pressure was normal during the first catheterization procedure. The secretion rate of the tumour was not high enough to produce a distinct increase in plasma catechol amine concentration. In the presence of a tumour with very little secretion, such as this one, the catheterization procedure above proved to be of no assistance in localizing the neoplasm.

The second catheterization procedure performed in connection with a positive histamine-test provided unequivocal evidence of a tumour in the upper abdomen and supplied information concerning the involved side.

Table 17. *Daily urinary excretion of adrenaline and noradrenaline* in Case No. 1.*

Date	Adrenaline µg/24 hr	Noradrenaline µg/24 hr	Operations
July 30 1958	1860	546	
Aug. 9	141	60	
11	99	39	
Sept. 30			no tumour found
Oct. 18	50	33	
Dec. 19	52	61	
Febr. 13 1959	84	50	
March 18	82	46	
April 24			phaeochromocytoma
May 6	6	45	removed
15	0	42	

* The method of determination of catechol amines in urine has been described by Carlsson, Rasmussen and Kristjansen (1959).

Case 2. E.H. A 56-year-old female was admitted to the Rigshospital of Copenhagen in February 1959 because of hypertension combined with episodes of excessive palpitations, perspiration, headache, vomiting, weakness and anxiety. These fits as a rule lasted for 15—30 minutes, occasionally as long as two hours, and the patient had suffered considerable discomfort for the last four years. She had dyspnea on exertion. On physical examination a systolic murmur was noted. The blood pressure ranged from 200/100 to 220/140 mm Hg. The electrocardiogram showed a considerable degree of left axis deviation and changes of the RS-T segments indicating left ventricular hypertrophy and strain. There were advanced retinal changes with hemorrhages and exudate. The fasting blood glucose was slightly elevated. The urine showed consistent presence of protein. Urinary excretion of adrenaline and noradrenaline showed increased values (Table 18). Retroperitoneal gas insufflation gave rise to suspicions that there might be a tumour at the site of the left suprarenal gland. In March an explorative incision was made, parallel with the twelfth rib for approach to the suprarenal gland. No tumour could be found on this occasion. In April the same incision was made on the right side, but without result.

As further surgical exploration was considered urgent the catheterization of the venae cavae was performed to localize the tumour. Since no significant amount of adrenaline was present in the urine, all possible sites had to be considered. The catheterization was performed through the right saphenous vein and the tip of the catheter was introduced into the superior vena cava. Blood samples were drawn during a single passage down the vena cava with duplicate specimens. The catechol amine content of all the plasma samples was increased, and the highest values were obtained in the lower part of the vena cava inferior. No

Table 18. *Daily urinary excretion of adrenaline and noradrenaline* in Case No. 2.*

Date	Adrenaline µg/24 hr	Noradrenaline µg/24 hr	Operations
Febr. 2 1959	97	1014	
March 3	66	454	
10			no tumour found
April 14			no tumour found
16	11	305	
June 24	44	617	
29			paraganglioma removed
Sept. 16	18	412	
Oct. 2			phaeochromocytoma removed
10	8	75	
29	9	36	

information as to which side was involved was obtained as only single samples (with the catheter tip in the centre of the vessel) were taken at various levels from the lower part of the inferior vena cava. The patient was subsequently explored through longitudinal abdominal incision and a tumour as large as a hazel nut was found, connected with the right ovary (microscopic diagnosis: paraganglioma). Postoperatively the patient did not report any more fits. Her blood pressure remained at a somewhat lower level, around 180/100. Her daily excretion of adrenaline and noradrenaline remained high. A new vena caval catheterization was decided, which was performed in October, 1959. The catheter was introduced through the left saphenous vein and its tip was lodged in the vena cava superior. The blood samples were drawn during a single passage down the venae cavae with duplicate specimens at different levels. The catechol amine content from all the plasma samples was increased, particularly in the lowest part of the vena cava inferior. See Figure 21. The data were interpreted as demonstrating a tumour in the lower part of the abdomen. The patient was surgically explored through an abdominal incision and a phaeochromocytoma as big as a golf ball was found in the lower part of the abdomen in the posterior wall of the bladder. Postoperatively the patient had no subjective discomfort, the blood pressure ranged between 150/90 and 160/100 mm Hg, the urinary excretion of catechol amines was normal.

In this case the blood pressure was sufficiently raised during the catheterization procedure to suggest that the tumour was secreting continuously. The plasma catechol amine concentrations were elevated

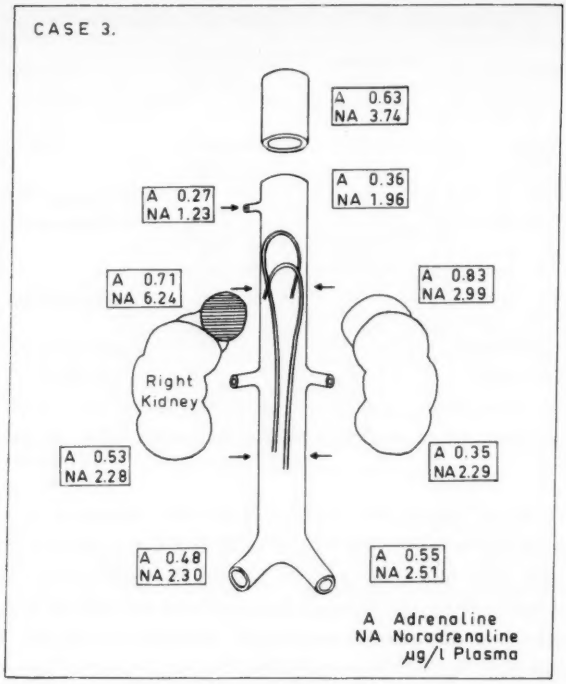


Figure 22.

in all blood samples drawn. On two occasions, the catheterization procedure here provided unequivocal evidence of a tumour in the lower abdomen.

Case 3. B.J. A 46-year-old male was admitted to the Rigshospital of Copenhagen in August 1959 because of fits of angina pectoris, palpitations and anxiety induced by physical exertion. When at rest, he never suffered any fits. The blood pressure ranged between 155/90 and 160/110 mm Hg. The general physical examination was otherwise negative. The urinary excretion of adrenaline was 31 µg and that of noradrenaline 1187 µg per 24 hours. In connection with the selective aortography the patient had a fit of the kind described above with a rise of blood pressure to 260 mm Hg systolically, which after 10 mg Regitin intravenously fell to 190 mm within two minutes. Aortography and retroperitoneal gas insufflation indicated an enlarged right-sided suprarenal gland. Intravenous urogram was normal. No marked difference in blood pressure was found during massage of each flank.

Catheterization was performed to evaluate the usefulness of the technique. Vena caval catheterization was performed through the right saphe-

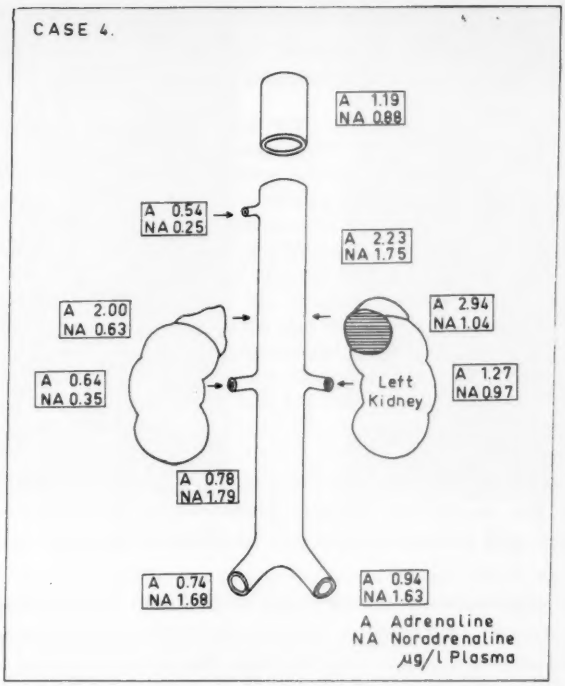


Figure 23.

nous vein and the catheter was advanced up into the superior vena cava. Blood samples were drawn during the passage down the venae cavae and duplicate specimens were obtained at various levels. The tip of the catheter was directed against the walls of the venae cavae on the respective side. The catechol amine content of all the plasma samples was increased. The specimens showed a sudden rise in catechol amine concentration at the site corresponding to the right suprarenal gland. See Figure 22. In October 1959 exploration of the right suprarenal area was performed through a flank incision. A phaeochromocytoma ($4 \times 2 \times 2$ cm) was found and removed from this region. The excised tumour contained 1 mg catechol amines per gram tissue, noradrenaline constituting 95 per cent of the total catechol amines present.

Though the catheterization was a supplementary procedure, the findings confirmed the presence of a tumour in the suprarenal region and afforded information on the side involved. Postoperatively a blood

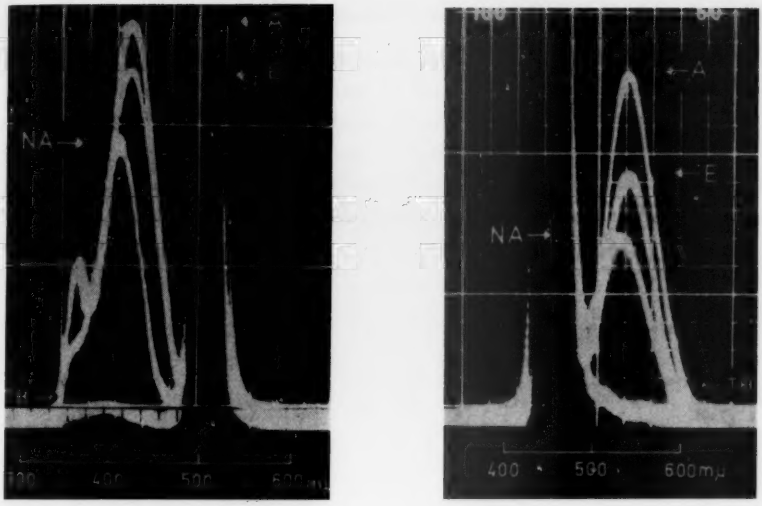
pressure of 150/100 mm was noted and the catechol amine excretion on two separate occasions subsequently was 12 and 2 μg adrenaline and 61 and 76 μg per 24 hours noradrenaline, respectively.

Case 4. E.P. A 44-year-old woman was admitted to the Rigshospital of Copenhagen in December 1959 because of hypertension. During the last four years the patient had suffered from headache and vertigo, and lately also by slight dyspnea on exertion. She had also recurrent episodes of palpitation and there was a progressive loss of weight during the last six months. A persistently high blood pressure was found with variations ranging from 200 to 250 systolically and from 120 to 125 diastolically with no drop induced by rest. Systolic murmur was heard over the apical region, the second aortic sound was accentuated. The electrocardiogram showed a considerable degree of left axis deviation and changes of the RS-T segments indicating left ventricular hypertrophy and strain. The retinal arterioles showed narrowing. The catechol amine excretion on two separate occasions was 473 μg and 422 μg adrenaline and 122 and 78 μg per 24 hours noradrenaline, respectively.

The high excretion of adrenaline suggested a tumour localized in the region of the suprarenal glands. Nonetheless, intravenous urogram, tomography and retroperitoneal gas insufflation showed normal conditions.

Surgical exploration was considered urgent and catheterization of the venae cavae was performed to localize the tumour pre-operatively. The cardiac catheter was introduced through the saphenous vein and passed up into the superior vena cava. Blood samples were drawn during the passage down the venae cavae and duplicate specimens were obtained at various levels with the tip of the catheter against the walls of the vena cava on the respective side. The catechol amine content of all the plasma samples was increased, the specimen at the site corresponding to the left suprarenal gland showing the highest concentration (Figure 23). The data were interpreted as demonstrating a tumour in the left suprarenal region of the upper abdomen. The patient was subsequently explored through an abdominal incision and a phaeochromocytoma was found in the left suprarenal region. The excised tumour weighed 17 gram and contained 4.9 mg adrenaline per gram tissue constituting no less than 95 per cent of the total catechol amines present. The urinary excretion of catechol amines showed normal values on two separate occasions after operation.

In this case the catheterization procedure provided evidence of a tumour in the upper abdomen and supplied information on the side involved.



I II

Figure 24. Activation and Fluorescence Spectra of an Eluate from a Blood Sample obtained from Peripheral Vein in a Case of Phaeochromocytoma.

I Activation spectrum. Fluorescence wave-length 540 mμ.
II Fluorescence spectrum. Activating wave-length 455 mμ.

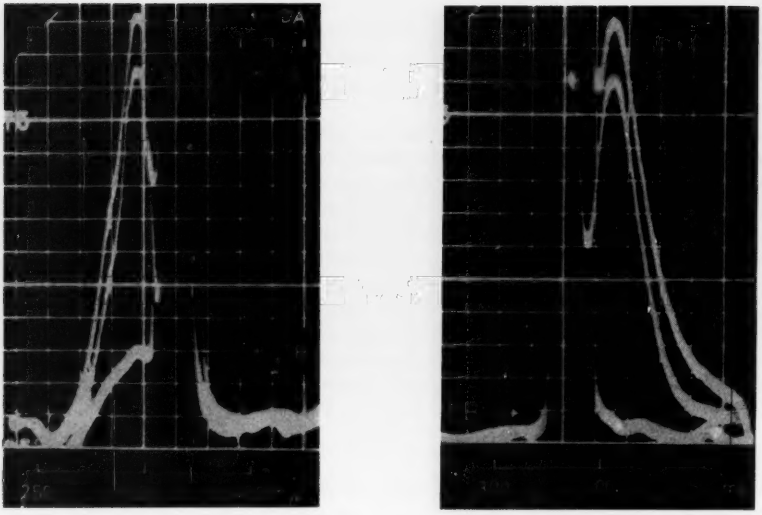
A Adrenaline standard.
NA Noradrenaline standard.
TB Faded sample blank.

The activation and fluorescence peaks from adrenaline are 425 and 545 mμ, respectively. The corresponding peaks for noradrenaline are at 410 and 535 mμ, respectively. The spectra of the eluate come close to those obtained from adrenaline.

Comment

The concentration of adrenaline in peripheral venous plasma obtained at rest in the above mentioned cases varied from 0.05 to 0.82 μg and noradrenaline from 0.35 to 2.64 μg per litre of plasma. The lowest values being found in Case No. 1 where they were withing the normal range.

The highest concentrations of catechol amines in the peripheral vein of these patients were found at operation, *e.g.* in Case No. 1 the highest value for adrenaline was 54 μg and for noradrenaline 35 μg per litre of plasma. The spectra obtained from this blood sample are demonstrated in Figure 24.



I II

Figure 25. Activation and Fluorescence Spectra of Dopamine and of an Eluate from a Blood Sample Drawn from the Antecubital Vein in a Case of Sympathicogonioma.

I Activation spectrum. Fluorescence wave-length 410 mμ.
II Fluorescence spectrum. Activating wave-length 345 mμ.

DA Dopamine standard.
E Eluate from plasma.
TB Faded sample blank.

Spectra obtained after oxidation and rearrangement in alkali. The activation and fluorescence peaks obtained from dopamine are 345 and 410 mμ, respectively.

During vena cava catheterization some blood samples were withdrawn from different points in the vascular tree, *inter alia* from the hepatic and left renal veins, and these samples were analyzed for dopamine. The presence of this substance could not be demonstrated in any of the subjects investigated. In one patient with sympathicogonioma, however, with a daily urinary excretion of 100 mg of dopamine, this substance was found in the peripheral vein. The concentration was 238 μg per litre of plasma. Figure 25 shows the fluorescence spectrum of the fluorophore of dopamine obtained from the blood sample.

Summary

The catheterization of the venae cavae proved to be useful in cases of phaeochromocytoma. In three out of four cases the venous blood from the tumour had a sufficiently high concentration of catechol amines to allow relatively precise estimation. A consistent elevation of the plasma level of catechol amines is not always found (Case No. 1) which suggests that some tumour may have paroxysmal secretion.

General Summary

A spectrophotofluorometric method based on the trihydroxyindole procedure for the determination of the minute amounts of catechol amines in human plasma is presented in this work.

The method possesses a satisfactory degree of precision and reproducibility as judged on the following basis:

1) Repeated analysis of duplicate samples of adrenaline and noradrenaline, mixed in various proportions and carried through the procedure, yielded quantitative recovery within a relatively narrow range.

2) The recoveries of adrenaline and noradrenaline added to plasma in varying amounts and carried out through the entire procedure were similarly consistent within limits which would permit the detection of minute changes in the concentration in the samples from physiological experiments.

3) Although the method is pushed to the limits of sensitivity it yielded reproducible values as judged on the concentrations of adrenaline and noradrenaline in blood samples from the different parts of the circulation in the human body.

4) Experimental indication of the specificity of the trihydroxyindole method is afforded by the results obtained at intravenous infusions of catechol amines. The findings indicate the ability to differentiate between adrenaline and noradrenaline.

The concentrations of adrenaline and noradrenaline in blood samples drawn from the different parts of the circulation in the human body are very low. The values in peripheral venous plasma obtained in the present work (0.07 μg of adrenaline and 0.35 μg of noradrenaline per litre of plasma with standard deviations of 0.06 and 0.10, respectively) are in good agreement with those obtained by VALK and PRICE (1956) and COHEN and GOLDENBERG (1957) who use the trihydroxyindole method but much lower than those obtained by the ethylenediamine condensation method of WEIL-MALHERBE and BONE (1954, 1958).

The findings with respect to the relative concentrations of catechol amines in platelets and in plasma indicate that while the concentration in platelets is higher than in plasma, the contribution of platelet-bound amines to the total in platelet-rich plasma is insignificant.

It is reasonable to conclude that adrenaline is secreted into blood entering the upper part of vena cava and disappears in the peripheral tissue (positive arterio-venous difference). It is also reasonable to assume that noradrenaline is released by the adrenergic nerve endings. The lowest values obtained from blood samples from the hepatic vein support the hypothesis that the organs in the region of the portal circulation are able to metabolize catechol amines.

The effect of insulin hypoglycemia on plasma concentrations of catechol amines showed that the changes in the blood sugar level were followed almost instantaneously by complementary changes in the level of adrenaline, while the level of noradrenaline did not alter to any marked extent.

Constant rate infusions of adrenaline and noradrenaline were characterized by the attainment of a steady state concentration within $7\frac{1}{2}$ to 10 minutes. Steady state concentrations were proportional to the infusion speeds. Noradrenaline attained a higher concentration in plasma for comparable infusion speeds than did adrenaline.

The half-life in plasma of the infused noradrenaline was 2.3 minutes (corresponding with a turnover time of $3\,{}^{1}/_{3}$ minutes).

Normal resting secretion rate of 0.018 μg of noradrenaline per kg/min. and 0.008 μg of adrenaline per kg/min. were calculated from the data.

Plasma concentrations of adrenaline during work test with successively increased loads showed a small increase in the level of adrenaline. In marked contrast to this the levels of noradrenaline increased rapidly and exponentially with the load; the rapid increase in concentration as the load was increased provides support for the "overflow" hypothesis of CANNON and ROSENBLUETH (1937, 1949; ROSENBLUETH, 1950) in which it was proposed that effector substances released at adrenergic nerve endings may under certain circumstances escape into the blood stream. It is not clear why the level of noradrenaline increases so rapidly at the higher loads but the form of the curve is suggestive of saturation of enzyme systems destroying noradrenaline and situated very near the sites of production in the peripheral tissues.

The experiments involving catheterization of the left renal vein indicate that there is no major contribution by the suprarenal glands to the increased amounts of noradrenaline secreted during excercise. Enhanced peripheral release of noradrenaline during excercise is generalized throughout the body rather than localized phenomenon confined to the venous blood returning from regions of the body where the work is being done.

The studies on the effect of body posture in healthy subjects and in

subjects with arterial orthostatic anemia on the concentration of adrenaline and noradrenaline in plasma showed that the noradrenaline release is an important factor in the homeostatic mechanism. The basic involvement of a sympathicotonic, *i.e.* neurosecretory, deficiency was obvious in the case of postural hypotension.

Measurement of the concentration of catechol amines at different levels of the venae cavae was of use in diagnosing doubtful cases of phaeochromocytoma as well as an aid to the surgeon by the localization of the tumour. Catheterization was employed in four cases and the method was found to be valuable. By careful sampling at various levels with the tip of the catheter directed against the walls of the vena cava it was possible to lateralize the tumour in three cases.

Acknowledgement

The investigation was supported by a grant from the Medical Faculty of the University of Lund.

APPENDIX

Table. 1. *Effect of insulin (0.1 I.U./kg bodyweight)*

Case No.	Age	Sex	Resting			Insulin period (minutes after injection)				
						10 min.			20 min.	
			Pulse	Blood pressure		Pulse	Blood pressure		Pulse	Blood
				systolic	diastolic		systolic	diastolic		systolic
1	22	F	62	115	70	62	115	65	64	105
2	41	F	70	110	70	66	110	70	68	110
3	29	F	80	120	65	80	120	60	96	110
4	23	F	84	125	80	80	125	80	88	135
5	21	F	68	115	70	80	115	75	92	125
6	19	F	88	145	65	84	145	75	120	160
7	33	F	64	110	80	72	120	85	68	115
8	39	F	84	115	80	84	120	80	80	115
9	27	F	60	105	75	60	105	65	61	100
10	20	F	64	120	70	68	120	70	84	115
11	21	F	92	135	75	94	135	70	100	130
12	61	F	66	120	85	70	115	70	72	125
13	68	F	84	130	70	84	130	70	84	120
14	25	M	64	140	80	64	140	70	64	130
15	30	M	68	125	75	68	115	75	69	120
16	22	M	44	140	90	48	145	80	88	150
17	44	M	76	115	75	76	110	75	72	110
18	26	M	76	130	85	76	130	80	72	135
19	25	M	72	120	85	68	120	70	72	120
20	23	M	68	130	85	66	135	90	92	145
21	26	M	68	120	80	76	125	85	72	125
22	30	M	72	140	90	72	135	90	72	130
23	27	M	58	120	70	58	120	70	62	125
24	33	M	76	150	90	76	140	75	92	145
25	23	M	76	135	65	76	135	70	84	135
26	41	M	58	110	80	58	105	70	58	105
27	38	M	92	130	70	96	125	70	100	125
28	23	M	66	140	80	60	130	60	62	130
29	23	M	82	150	70	84	155	70	96	155
30	34	M	72	130	70	74	130	70	78	130
31	39	M	60	110	65	60	105	60	62	105
Mean			71	126	76	72	125	73	79	125
S.E.			±2.0	±2.2	±1.4	±2.0	±2.3	±1.4	±2.7	±2.7
n			31	31	31	31	31	31	31	31

on pulse rate (per min.) and blood pressure (mm Hg).

Insulin period (minutes after injection)									
20 min.	30 min.			45 min.			90 min.		
pressure	Pulse	Blood pressure		Pulse	Blood pressure		Pulse	Blood pressure	
diastolic		systolic	diastolic		systolic	diastolic		systolic	diastolic
65	72	110	65	84	105	50	68	105	70
75	86	110	60	82	110	65	69	110	70
50	104	110	35	86	110	40	76	110	65
80	88	125	90	88	125	75	76	130	70
70	108	135	60	70	120	55	76	115	60
50	96	145	40	92	140	45	76	130	65
70	76	115	65	66	115	65	62	110	70
80	88	115	65	83	110	70	76	115	80
65	80	105	55	67	110	45	60	95	65
50	86	125	60	68	120	65	64	120	70
70	104	125	60	82	130	60	84	130	70
70	82	125	65	68	110	60	64	105	60
60	85	115	60	81	115	60	80	115	60
75	68	135	60	60	135	55	60	130	80
70	88	115	40	90	155	50	68	125	60
75	88	170	70	80	165	65	64	145	85
75	88	105	65	76	110	65	72	100	70
95	78	140	75	72	135	60	74	130	90
70	88	125	60	72	115	60	68	115	60
80	92	150	70	84	155	75	64	145	90
75	112	130	70	72	110	65	68	120	80
75	88	155	55	68	140	70	70	130	80
70	68	125	70	82	130	60	56	105	65
75	84	145	65	80	130	70	68	130	70
70	96	150	50	80	145	60	72	135	70
70	78	130	75	68	130	75	58	100	70
60	90	120	60	88	120	60	88	125	70
60	72	140	45	62	135	50	56	130	70
60	96	160	55	84	150	60	72	145	70
65	84	130	50	75	120	55	76	120	70
65	82	110	50	66	110	45	65	105	60
69	87	129	60	77	126	60	69	120	70
±1.7	±1.9	±3.0	±2.1	±1.6	±2.9	±1.7	±1.4	±2.5	±1.5
31	31	31	31	31	31	31	31	31	31

Table 2. *Effect of insulin (0.1 I.U./kg bodyweight) on the plasma levels (μg/lit*

Case No.	Age	Sex	Resting			Insulin period (minutes after injection)									2 m		30 m	
						5 min.	10 min.			20 min.								
			Blood sugar	A	NA	Blood sugar	Blood sugar	A	NA	Blood sugar	A	NA	Blood sugar	A	Bl su	od gar	A	
1	22	F	0.086	0.10	0.42	0.086	0.078	0.09	0.22	0.050	0.13	0.32	0.0		32		0.3	
2	41	F	0.120	0.34	0.31	0.118	0.096	0.02	0.36	0.067	0.06	0.22	–		56		1.1	
3	29	F	0.080	0.10	0.24	0.090	0.074	0.05	0.23	0.050	0.28	0.21	-		44		1.0	
4	23	F	0.100	0.07	0.25	0.098	0.090	0.15	0.28	0.059	0.31	0.29	0.0		46		0.3	
5	21	F	0.102	0.07	0.42	0.088	0.075	0.27	0.34	0.059	0.30	0.41	0.0		65		0.9	
6	19	F	0.086	0.03	0.17	0.080	0.065	0.04	0.19	—	—	—	–		63		0.5	
7	33	F	0.110	0.22	0.43	0.094	0.084	0.24	0.59	0.063	0.31	0.76	0.0		59		1.3	
8	39	F	0.094	0.03	0.36	0.084	0.065	0.05	0.40	0.061	0.05	0.41	–		44		0.3	
9	27	F	0.084	–0.02	0.30	0.078	0.074	0.42	0.24	0.051	0.30	0.36	–		42		0.8	
10	20	F	0.090	0.09	0.38	0.090	0.072	0.23	0.40	0.051	0.28	0.33	0.0		66		0.5	
11	21	F	0.093	0.13	0.57	0.091	0.081	0.20	0.48	0.050	0.38	0.44	0.0		56		0.8	
12	61	F	0.094	0.01	0.31	0.082	0.052	0.01	0.32	0.044	0.09	0.30	–		40		0.7	
13	68	F	0.100	0.00	0.32	0.100	0.096	0.16	0.20	0.076	0.24	0.21	—		69		0.5	
14	25	M	0.110	0.03	0.31	0.090	0.080	0.03	0.29	0.060	0.33	0.14	–		60		0.7	
15	30	M	0.102	1.10	0.54	0.100	0.069	0.09	0.44	0.050	0.24	0.58	–		50		0.2	
16	22	M	0.106	0.02	0.50	0.086	0.070	0.01	0.38	0.055	0.43	0.14	0.0		57		1.0	
17	44	M	0.099	0.06	0.39	0.093	0.081	0.21	0.39	0.066	0.14	0.35			62		0.4	
18	26	M	0.112	0.15	0.47	0.102	0.092	0.18	0.44	0.063	0.42	0.56	0.		57		0.5	
19	25	M	0.084	0.19	0.34	0.077	0.067	0.06	0.36	0.036	0.24	0.08	0.		48		0.7	
20	23	M	0.109	0.14	0.47	0.099	0.087	0.19	0.32	0.045	0.50	0.16	0.0		49		0.5	
21	26	M	0.099	0.02	0.27	0.091	0.062	0.10	0.49	0.036	0.06	0.37	+		61		1.0	
22	30	M	0.086	0.07	0.38	0.084	0.072	0.03	0.30	0.064	0.06	0.25	-		55		0.5	
23	27	M	0.103	0.19	0.47	0.097	0.081	0.20	0.71	0.058	0.35	0.47	0.		52		0.3	
24	33	M	0.096	–0.01	0.39	0.080	0.076	0.02	0.51	0.050	0.04	0.44	0		12		0.4	
25	23	M	0.111	0.08	0.42	0.103	0.078	0.17	0.42	0.055	0.09	0.44	0.0		066		1.0	
26	41	M	0.094	–0.04	0.16	0.082	0.074	0.06	0.13	0.047	0.06	0.13	–		038		1.1	
27	38	M	0.096	0.05	0.63	0.094	0.074	0.08	0.50	0.044	0.27	0.65	—		053		0.8	
28	23	M	0.113	–0.01	0.48	0.092	0.080	–0.04	0.57	0.066	0.16	0.63	–		058		0.	
29	23	M	0.106	0.10	0.46	0.096	0.078	0.11	0.49	0.049	0.29	0.27	0.0		066		1.1	
30	34	M	0.091	0.09	0.33	0.085	0.085	0.14	0.30	0.062	0.54	0.16	-		060		0.4	
31	39	M	0.100	0.01	0.32	0.086	0.078	0.08	0.38	0.065	0.42	0.37	–		057		0	
Mean			0.099	0.07	0.38	0.091	0.077	0.12	0.38	0.055	0.25	0.35	0.0		054		0.	
S.E.			±0.002	±0.01	±0.02	±0.002	±0.002	±0.02	±0.02	±0.002	±0.03	±0.03	±0.		0.002		±	
n			31	31	31	31	31	31	31	30	30	30	1.		31		3	

* All values of catechol amines given in this book are uncorrected for recoveries.

(μg/litre) *naline (A) and noradrenaline (NA)* and blood sugar (g/100 ml) in healthy subjects.*

		Insulin period (minutes after injection)											
	2 m	30 min.		35 min.	45 min.			60 min.	90 min.			120 min.	180 min.
A	Bl su	Blood sugar	A	NA	Blood sugar	Blood sugar	A	NA	Blood sugar	Blood sugar	A	NA	Blood sugar	Blood sugar
2	0.(	032	0.37	0.12	0.050	0.053	0.34	0.09	0.071	0.080	0.10	0.24	—	—
2	-	056	1.10	0.24	—	0.058	0.21	0.27	0.067	0.084	–0.03	0.29	—	—
1	-	044	1.08	0.07	—	0.061	0.45	0.21	0.065	0.076	0.00	0.17	0.082	0.082
9	0.(	046	0.33	0.15	0.061	0.071	0.36	0.12	0.106	0.090	0.15	0.36	—	—
1	0.(	065	0.98	0.78	0.075	0.084	0.53	0.11	0.090	0.114	0.22	0.24	—	—
	–	063	0.53	0.30	—	0.073	0.69	0.11	0.073	0.080	0.26	0.27	0.094	0.092
6	0.(	059	1.33	1.06	0.069	0.080	0.46	0.93	0.086	0.096	0.37	0.41	—	—
	–	044	0.37	0.31	—	0.066	0.23	0.29	0.078	0.076	0.06	0.31	—	—
	–	042	0.81	0.21	—	0.066	0.48	0.14	0.084	0.090	–0.04	0.15	0.088	0.084
	0.(	066	0.55	0.16	0.068	0.070	0.32	0.16	0.072	0.080	0.32	0.21	—	—
	0.(	056	0.82	0.50	0.062	0.069	0.43	0.57	0.073	0.081	0.34	0.49	—	—
	–	040	0.79	0.18	—	0.055	0.49	0.13	0.076	—	0.28	0.21	—	—
	–	069	0.53	0.34	—	0.073	0.26	0.24	0.077	0.084	0.10	0.22	—	—
	–	060	0.74	0.60	—	0.070	0.58	0.16	0.080	0.100	0.57	0.33	—	—
	–	050	0.27	0.66	—	0.073	1.03	0.78	0.073	0.077	0.02	0.61	—	—
	0.0	057	1.01	0.13	0.059	0.059	0.96	0.14	0.067	0.070	0.30	0.28	—	—
		062	0.49	0.23	—	0.085	0.47	0.22	0.089	0.091	0.01	0.33	—	—
	0.	057	0.58	0.28	0.077	0.098	0.44	0.56	0.100	0.104	0.20	0.27	—	—
	0.	048	0.76	0.25	0.050	0.063	0.56	0.30	0.065	0.071	0.21	0.28	—	—
	0.(	049	0.55	0.15	0.053	0.053	1.11	0.08	0.091	0.099	0.39	0.46	—	—
	–	061	1.05	0.28	—	0.070	0.95	0.79	0.087	0.099	0.46	0.43	—	—
	-	055	0.50	0.21	—	0.072	0.40	0.29	0.070	0.084	0.05	0.31	—	—
	0.	052	0.32	0.43	0.053	0.062	0.53	0.35	0.075	0.081	0.36	0.41	—	—
		42	0.49	0.19	—	0.072	0.26	0.12	0.056	0.076	0.09	0.21	0.090	0.088
	0.0	066	1.05	0.44	0.078	0.085	0.39	0.28	0.087	0.095	0.35	0.41	—	—
	–	038	1.11	0.23	—	0.064	0.23	0.53	0.066	0.074	0.02	0.27	0.096	0.098
	—	053	0.81	0.60	—	0.055	0.81	0.89	0.065	0.080	0.66	0.78	—	—
	–	058	0.74	0.79	—	0.090	0.50	0.74	0.090	0.090	0.33	0.56	—	—
	0.(	066	1.57	0.29	0.066	0.070	0.70	0.35	0.084	0.096	0.19	0.46	—	—
	-	060	0.41	0.34	—	0.081	0.23	0.28	0.085	0.091	0.04	0.34	—	—
	–	057	0.48	0.30	—	0.078	0.08	0.36	0.078	0.080	0.05	0.27	0.084	0.104
	0.0	054	0.73	0.35	0.063	0.070	0.50	0.34	0.078	0.086	0.21	0.34	0.089	0.091
	±0.(	0.002	±0.06	±0.04	±0.003	±0.002	±0.05	±0.05	±0.002	±0.002	±0.03	±0.02	±0.002	±0.003
	1.	31	31	31	31	31	31	31	31	31	31	31	6	6

Table 3. *Plasma levels (μg/litre) of adrenaline (A) and nor-*

I. *Healthy*

Case No.	Age	Sex	Antecubital vein		Femoral artery		Pulmonary artery		Inferior (upper
			A	NA	A	NA	A	NA	A
1	16	M	0.22	0.29	0.23	0.30	0.22	0.31	0.24
2	27	M	0.13	0.19	0.17	0.21	0.24	0.21	0.27
3	41	M	0.02	0.26	–	–	0.27	0.25	0.42
4	19	M	0.23	0.41	0.32	0.45	0.28	0.45	0.74
5	26	M	0.00	0.24	–	–	0.10	0.14	0.23
6	21	M	0.09	0.30	–	–	–	–	0.36
7	38	M	0.08	0.38	–	–	–	–	0.33
8	25	M	0.32	0.55	–	–	–	–	–
9	27	M	0.32	0.53	–	–	–	–	–
10	23	M	0.05	0.35	–	–	–	–	–
11	27	M	0.11	0.40	–	–	–	–	–
			0.05	0.34	–	–	–	–	–
12	23	M	0.09	0.40	–	–	–	–	–
			0.11	0.49	–	–	–	–	–
13	28	M	0.07	0.27	–	–	–	–	–
14	38	M	0.33	0.54	–	–	–	–	0.52
Mean			0.14	0.36	0.24	0.32	0.22	0.27	0.39
S.E.			±0.03	±0.03	±0.04	±0.07	±0.03	±0.05	±0.06
n			16	16	3	3	5	5	8

adrenaline (NA) in different sites of blood flow in the body.

subjects.

vena cava part)	Inferior vena cava (lower part)		Hepatic vein		Left renal vein		Right renal vein	
NA	A	NA	A	NA	A	NA	A	NA
0.40	0.11	0.26	–	–	–	–	–	–
0.24	–	–	0.00	0.00	–	–	–	–
0.21	0.01	0.32	0.11	0.27	0.43	0.17		
0.57	0.24	0.30	–	–	–	–	0.36	0.33
0.13	0.09	0.20	—0.02	0.06	–	–	–	–
0.18	0.19	0.25	0.00	0.00	0.35	0.11	0.16	0.30
0.19	0.19	0.45	0.14	0.13	–	–	–	–
–	–	–	0.04	0.40	–	–	–	–
–	–	–	0.06	0.14	–	–	–	–
–	–	–	0.01	0.12	–	–	–	–
–	–	–	–	–	0.44	0.20	–	–
–	–	–	–	–	0.46	0.19	–	–
–	–	–	–	–	0.27	0.25	–	–
–	–	–	–	–	0.28	0.26	–	–
–	–	–	0.14	0.13	0.33	0.14	–	–
0.39	–	–	–	–	0.30	0.13	–	–
0.29 ±0.05 8	0.14 ±0.03 6	0.30 ±0.04 6	0.05 ±0.02 9	0.14 ±0.04 9	0.36 ±0.03 8	0.18 ±0.02 8	0.26 ±0.10 2	0.32 ±0.02 2

II. a) *Subjects with mitral, tricus-*
Normal pressure values by

Case No.	Age	Sex	Antecubital vein		Femoral artery		Pulmonary artery		Inferior vena cava (upper part)	
			A	NA	A	NA	A	NA	A	NA
15	32	F	0.12	0.28	–	–	–	–	0.27	0.25
16	27	M	0.14	0.33	0.23	0.41	0.17	0.15	–	–
17	26	M	0.13	0.30	0.09	0.47	0.13	0.54	0.33	0.07
18	32	M	–	–	–	–	0.31	0.20	–	–
19	41	M	–0.01	0.40	–	–	0.18	0.17	0.30	0.18
20	43	F	0.10	0.24	0.16	0.24	0.14	0.18	0.36	0.15
21	48	M	0.07	0.42	0.23	0.30	0.18	0.25	0.56	0.36
22	42	M	0.10	0.36	0.19	0.18	–	–	–	–
23	39	F	0.19	0.33	0.15	0.17	0.25	0.06	0.28	0.23
24	39	M	0.16	0.27	–	–	–	–	0.36	0.28
Mean			0.11	0.34	0.18	0.30	0.19	0.22	0.35	0.21
S.E.			±0.02	±0.02	±0.02	±0.05	±0.02	±0.06	±0.04	±0.04
n			9	9	6	6	7	7	7	7

b) *Subjects with congenital heart*
Normal pressure values by

Case No.	Age	Sex	Antecubital vein		Femoral artery		Pulmonary artery		Inferior vena cava (upper part)	
			A	NA	A	NA	A	NA	A	NA
25	26	F	0.11	0.34	–	–	–	–	0.33	0.23
26	19	F	0.15	0.13	0.20	0.12	0.37	0.19	0.35	0.18
27	27	F	0.05	0.35	–	–	–	–	0.48	0.39
28	24	F	–0.02	0.28	0.27	0.21	0.18	0.12	0.21	0.12
29	45	F	0.11	0.69	–	–	–	–	0.35	0.25
30	25	F	–0.02	0.26	–	–	–	–	0.43	0.11
Mean			0.06	0.34	0.24	0.16	0.27	0.15	0.36	0.21
S.E.			±0.03	±0.08	±0.03	±0.04	±0.10	±0.03	±0.04	±0.04
n			6	6	2	2	2	2	6	6

pid and aortic valvular disease.
right-heart catheterization

Inferior vena cava (lower part)		Hepatic vein		Left renal vein		Coronary sinus		Left atrium	
A	NA	A	NA	A	NA	A	NA	A	NA
–	–	0.08	0.19	–	–	0.17	0.39	–	–
–	–	0.22	0.50	2.19	0.32	–	–	–	–
–	–	–	–	–	–	–	–	–	–
–	–	0.10	0.13	–	–	–	–	–	–
–	–	0.16	0.22	–	–	–	–	–	–
–	–	0.00	0.00	–	–	0.14	0.08	–	–
0.12	0.39	0.00	0.00	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–
0.04	0.36	0.05	0.12	–	–	–	–	–	–
0.12	0.18	0.13	0.19	–	–	–	–	–	–
0.09	0.31	0.09	0.17	2.19	0.32	0.15	0.23	–	–
±0.03	±0.07	±0.03	±0.06	–	–	±0.01	±0.15		
3	3	8	8	1	1	2	2		

disease (left-to-right shunts).
right- and left-heart catheterization.

Inferior vena cava (lower part)		Hepatic vein		Left renal vein		Coronary sinus		Left atrium	
A	NA	A	NA	A	NA	A	NA	A	NA
0.10	0.24	–	–	–	–	–	–	0.17	0.19
0.15	0.18	0.00	0.00	–	–	–	–	–	–
0.06	0.38	–	–	4.37	1.41	–	–	0.17	0.22
0.05	0.32	–0.06	0.25	–	–	–	–	–	–
–	–	–	–	2.20	0.53	–	–	–	–
–	–	0.16	0.01	2.85	0.84	–	–	0.12	0.19
0.09	0.28	0.03	0.09	3.14	0.92	–	–	0.15	0.20
±0.02	±0.04	±0.07	±0.08	±0.64	±0.26			±0.02	±0.01
4	4	3	3	3	3			3	3

III. *Subjects with mitral, tricuspid and aortic valvular disease.*

Case No.	Age	Sex	Antecubital vein		Femoral artery		Pulmonary artery		Inferior vena cava (upper part)		Inferior cava part)
			A	NA	A	NA	A	NA	A	NA	A
31	39	F	0.00	0.22	–	–	0.11	0.09	–	–	–
32	55	F	0.02	0.22	0.17	0.13	0.25	0.30	0.26	0.11	–
			0.17	0.16	–	–	–	–	–	–	–
33	47	M	0.19	0.20	0.12	0.11	0.24	0.14	0.31	0.18	–
34	35	F	0.11	0.37	–	–	0.17	0.16	–	–	–
35	42	F	–0.04	0.38	0.21	0.28	0.28	0.18	0.22	0.20	0.03
36	41	F	0.14	0.33	–	–	0.20	0.26	0.25	0.14	–
37	24	F	0.02	0.23	0.29	0.16	–	–	0.29	0.17	–
			–0.02	0.27	–	–	–	–	–	–	–
38	47	F	–	–	–	–	–	–	–	–	–
39	18	F	–	–	–	–	0.09	0.12	–	–	–
40	46	M	–0.10	0.41	–	–	0.19	0.45	0.31	0.27	–
			–0.02	0.28	–	–	–	–	–	–	–
41	49	M	0.03	0.27	–	–	–	–	0.35	0.10	0.01
42	56	M	0.00	0.23	–	–	0.21	0.28	0.43	0.35	0.06
43	36	M	0.03	0.31	–	–	–	–	0.26	0.07	0.07
			0.03	0.34	–	–	–	–	–	–	–
44	33	M	0.04	0.19	–	–	0.31	0.36	1.45	0.34	0.01
45	53	M	–0.05	0.66	0.19	0.54	0.22	0.59	0.62	0.58	–
			–0.11	0.65	–	–	–	–	–	–	–
46	45	F	–	–	–	–	0.36	0.33	0.87	0.26	–
47	56	M	0.02	0.29	–0.05	0.21	0.25	0.19	0.43	0.35	–
48	50	F	0.03	0.29	0.12	0.28	0.15	0.26	–	–	–
49	42	M	–	–	–	–	–	–	0.57	0.60	–
50	36	M	0.12	0.29	0.80	0.45	–	–	0.87	0.44	–
51	44	F	–0.04	0.17	0.10	0.22	–	–	–	–	–
52	52	M	–0.03	0.51	–	–	0.21	0.25	0.52	0.17	–
53	52	F	0.09	0.35	–	–	0.18	0.24	0.87	0.73	–
54	57	M	0.17	0.26	0.15	0.24	0.19	0.17	0.40	0.17	0.01
			0.17	0.41	–	–	–	–	–	–	–
55	44	M	0.17	0.29	0.33	0.22	0.28	0.16	0.51	0.21	0.23
56	43	F	–0.02	0.30	–	–	–	–	0.39	0.45	0.03
57	44	M	0.03	0.29	–	–	0.14	0.08	0.42	0.24	–
			0.03	0.32	–	–	–	–	–	–	–
58	45	F	0.14	0.34	–	–	0.19	0.14	0.32	0.19	–
59	56	F	0.18	0.46	–	–	–	–	–	–	–
Mean			0.05	0.32	0.22	0.26	0.21	0.24	0.50	0.29	0.05
S.E.			±0.02	±0.02	±0.07	±0.04	±0.01	±0.03	±0.06	±0.04	±0.03
n			32	32	11	11	20	20	22	22	8

Elevated pressure values by right-heart catheterization.

vena (lower	Hepatic vein		Left renal vein		Right renal vein		Coronary sinus		Left pulmonary vein	
NA	A	NA	A	NA	A	NA	A	NA	A	NA
–	–	–	–	–	–	–	–	–	–	–
–	0.02	0.05	–	–	–	–	0.13	0.53	–	–
–	–	–	–	–	–	–	–	–	–	–
–	0.00	0.00	–	–	–	–	0.02	0.21	–	–
–	–	–	–	–	–	–	–	–	–	–
0.26	–	–	–	–	–	–	0.15	0.46	–	–
–	0.10	0.13	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	0.07	0.17	–	–	–	–	0 05	0.57	–	–
–	–	–	–	–	–	–	–	–	0.01	0.18
–	–	–	–	–	–	–	0.35	0.78	–	–
–	–	–	–	–	–	–	–	–	–	–
0.25	0.12	0.11	–	–	–	–	–	–	–	–
0.37	–	–	–	–	–	–	–	–	–	–
0.28	0.00	0.00	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
0.13	0.00	0.74	–	–	–	–	–	–	–	–
–	0.00	0.00	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	0.11	0.27	–	–	–	–	–	–	–	–
–	–0.02	0.16	–	–	–	–	0.29	0.47	–	–
–	–	–	–	–	–	–	–	–	–	–
–	0.12	0.49	–	–	–	–	0.16	1.03	–	–
–	0.00	0.00	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	0.00	0.00	–	–	–	–	–	–	–	–
–	–	–	–	–	0.03	0.36	–	–	–	–
0.37	0.06	0.15	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
0.31	0.07	0.16	–	–	–	–	–	–	–	–
0.28	0.01	0.15	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	0.11	0.17	0.08	0.20	–	–	–	–	–	–
–	–	–	0.55	0.33	–	–	–	–	–	–
0.28 ±0.03 8	0.05 ±0.01 17	0.16 ±0.05 17	0.32 ±0.23 2	0.27 ±0.06 2	0.03 – 1	0.36 – 1	0.16 ±0.05 7	0.58 ±0.10 7	0.01 – 1	0.18 – 1

Table 4. *Pulse rate (per min.) and systolic blood pressure (mm Hg) in healthy men at rest and during muscular work with successively increased loads.*

Case No.	Age	Resting		During work								5′ after work		10′ after work	
				300 kgm/min. 6′		600 kgm/min. 6′		900 kgm/min. 6′		1200 kgm/m. 6′					
		Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure
1	21	82	125	145	155	172	165	–	–	–	–	91	120	87	120
2	23	59	125	113	180	150	210	–	–	–	–	82	145	73	135
Mean		71	125	129	168	161	188	–	–	–	–	87	133	80	128
S.E.		±11.5	±0.0	±16.0	±12.5	±11.0	±22.5	–	–	–	–	±4.5	±12.5	±7.0	±12.5
n		2	2	2	2	2	2					2	2	2	2
3	29	71	125	109	155	147	205	185	205	–	–	107	135	107	130
4	30	48	135	107	155	130	170	160	190	–	–	63	130	69	130
5	20	73	125	107	145	132	160	174	180	–	–	77	135	77	130
6	17	66	115	115	155	143	160	150	160	–	–	74	110	71	110
7	20	59	125	100	150	130	165	161	185	–	–	83	125	72	120
8	40	78	135	109	170	133	175	165	210	–	–	99	145	96	155
9	24	58	130	85	150	117	175	148	200	–	–	69	135	60	125
10	29	59	120	104	125	127	155	170	175	–	–	74	125	69	115
11	28	53	125	87	145	130	180	174	210	–	–	86	140	70	135
12	21	60	115	96	160	120	180	132	200	–	–	64	115	–	–
13	29	46	140	79	175	104	190	136	220	–	–	59	150	51	145
14	20	64	120	115	150	150	155	174	175	–	–	89	140	83	130
15	23	62	120	87	155	109	180	143	200	–	–	69	125	64	120
16	31	54	120	80	120	109	155	155	170	–	–	78	120	75	115
17	34	56	105	104	130	136	160	175	175	–	–	82	120	86	110
18	32	63	120	107	135	129	155	170	175	–	–	90	125	88	125
19	30	57	125	94	150	120	180	142	205	–	–	60	140	59	125
20	25	69	135	95	145	114	170	160	185	–	–	73	130	66	130
21	19	62	115	95	145	130	165	170	165	–	–	74	115	78	115
												107	–	99	120

20	25	69	135	95	145	114	170	160	185	–	–	73	130	66	130
21	19	62	115	95	145	130	165	170	165	–	–	74	115	78	115
22	26	85	125	122	155	133	165	160	180	–	–	107	–	99	120
23	21	50	140	105	165	140	185	179	205	–	–	99	150	76	150
Mean		62	125	100	149	128	171	161	189	–	–	80	131	76	127
S.E.		±2.1	±1.9	±2.6	±3.0	±2.7	±2.9	±3.2	±3.7	–	–	±3.2	±2.6	±3.2	±2.8
n		21	21	21	21	21	21	21	21	–	–	20	20	20	20
24	24	51	110	91	120	118	140	150	160	173	180	67	125	68	115
25	29	57	120	78	135	102	165	128	190	155	215	77	130	64	120
26	21	65	125	104	160	122	180	143	190	173	195	86	130	77	130
27	37	65	135	109	150	122	160	140	170	155	190	70	140	80	130
28	21	62	130	90	160	125	175	147	195	175	210	79	130	70	125
29	31	43	120	72	135	98	160	123	190	145	200	50	120	55	120
30	25	54	135	81	165	104	195	125	220	155	240	71	130	68	120
31	29	42	140	77	165	109	185	143	205	165	215	69	145	56	135
32	28	62	120	87	140	105	170	143	200	170	215	82	135	85	125
33	34	56	120	86	150	104	165	127	170	150	185	77	130	78	120
34	20	44	125	87	160	104	175	125	200	160	225	69	145	66	135
35	29	48	130	74	145	94	175	125	205	160	230	59	130	54	120
36	22	64	120	87	135	105	150	135	160	172	170	81	125	76	115
37	29	66	135	94	170	115	185	137	200	165	215	80	145	74	135
38	35	54	125	89	155	109	160	143	185	165	205	77	130	81	120
39	36	67	120	91	125	102	145	129	170	165	190	74	125	81	115
40	38	85	120	112	150	137	160	171	205	190	225	107	110	104	120
41	29	62	140	104	190	130	200	155	220	180	230	100	140	90	135
Mean		58	126	90	151	111	169	138	191	165	208	76	131	74	124
S.E.		±2.5	±2.0	±2.7	±4.1	±2.8	±3.8	±3.0	±4.4	±2.6	±4.6	±3.1	±2.2	±3.0	±1.7
n		18	18	18	18	18	18	18	18	18	18	18	18	18	18

Table 5. *Plasma levels (μg/litre) of adrenaline (A) and noradrenaline (NA) in healthy men at rest and during muscular work with successively increased loads.*

Case No.	Age	Resting		During work								5′ after work	
				300 kgm/min. 6′		600 kgm/min. 6′		900 kgm/min. 6′		1200 kgm/min. 6′			
		A	NA	A	NA	A	NA	A	NA	A	NA	A	NA
1	21	0.04	0.48	0.11	0.61	0.19	0.83	—	—	—	—	0.11	0.26
2	23	0.11	0.37	0.10	0.51	0.25	0.73	—	—	—	—	0.25	0.73
Mean		0.07	0.42	0.10	0.56	0.22	0.78	—	—	—	—	0.18	0.50
S.E.		±0.03	±0.05	±0.00	±0.05	±0.03	±0.05	—	—	—	—	±0.07	±0.24
n		2	2	2	2	2	2					2	2
3	29	0.03	0.36	0.16	0.47	0.27	1.00	0.33	2.03	—	—	0.10	0.85
4	30	0.09	0.21	0.11	0.27	0.22	0.51	0.34	0.95	—	—	0.25	0.33
5	20	0.02	0.24	0.12	0.41	0.19	0.90	0.21	1.63	—	—	0.13	0.20
6	17	0.04	0.43	0.05	0.61	0.11	0.86	0.29	1.14	—	—	—	—
7	20	0.07	0.27	0.01	0.37	0.19	0.71	0.05	1.27	—	—	0.12	0.28
8	40	0.19	0.22	0.07	0.28	0.07	0.48	0.25	0.92	—	—	0.15	0.14
9	24	0.21	0.25	0.13	0.45	0.34	0.66	0.30	0.84	—	—	0.25	0.22
10	29	0.07	0.26	0.08	0.33	0.17	0.65	0.38	1.13	—	—	—	—
11	28	0.05	0.24	0.11	0.56	0.22	0.84	0.32	1.47	—	—	0.16	0.35
12	21	0.02	0.43	0.01	0.66	0.11	0.73	0.12	1.09	—	—	—	—
13	29	0.20	0.23	0.19	0.32	0.28	0.66	0.38	0.79	—	—	0.28	0.16
14	20	0.12	0.41	0.10	0.53	0.19	0.76	0.25	1.41	—	—	—	—
15	23	0.01	0.36	0.06	0.39	0.18	0.70	0.20	0.75	—	—	—	—
16	31	0.05	0.33	0.11	0.49	0.02	0.60	0.17	0.98	—	—	—	—
17	34	0.02	0.28	0.05	0.33	0.00	0.58	0.13	1.58	—	—	0.20	0.23
18	32	−0.03	0.32	0.03	0.35	0.27	0.64	0.21	1.08	—	—	—	—
19	30	0.10	0.35	0.11	0.49	0.15	0.60	0.50	1.43	—	—	0.33	0.34
20	25	0.08	0.30	0.14	0.44	0.16	0.77	0.32	1.00	—	—	—	—
												0.70	0.45
								0.22	1.27	—	—		

19	30	0.10	0.35	0.11	0.49	0.15	0.60	0.50	1.43	—	—	—	—
20	25	0.08	0.30	0.14	0.44	0.16	0.77	0.32	1.00	—	—	0.33	0.34
21	19	0.19	0.25	0.14	0.57	0.31	0.86	0.22	1.27	—	—	0.70	0.45
22	26	0.05	0.28	0.31	0.45	0.16	0.68	0.41	1.16	—	—	0.06	0.30
23	21	0.00	0.41	0.14	0.52	0.19	0.96	0.18	1.19	—	—	0.20	0.61
Mean		0.08	0.31	0.11	0.44	0.18	0.72	0.27	1.20	—	—	0.23	0.34
S.E.		±0.02	±0.02	±0.01	±0.02	±0.02	±0.03	±0.02	±0.07	—	—	±0.04	±0.05
n		21	21	21	21	21	21	21	21			13	13
24	24	0.04	0.21	0.11	0.25	0.31	0.74	0.26	1.01	0.29	1.88	—	—
25	29	0.00	0.38	0.23	0.45	0.38	0.68	0.57	0.93	0.64	2.38	—	—
26	21	−0.02	0.25	0.06	0.42	0.05	0.61	0.19	0.89	0.34	1.25	—	—
27	37	0.11	0.27	0.08	0.33	0.23	0.43	0.27	0.74	0.20	1.30	—	—
28	21	0.02	0.29	0.06	0.36	0.20	0.48	0.18	0.89	0.42	1.63	—	—
29	31	0.05	0.24	0.03	0.38	0.11	0.55	0.18	0.67	0.09	1.73	—	—
30	25	0.24	0.31	0.24	0.56	0.37	0.71	0.41	1.11	0.40	1.84	—	—
31	29	0.16	0.28	0.24	0.35	0.28	0.55	0.26	1.01	0.50	1.45	—	—
32	28	0.03	0.32	0.09	0.46	0.16	0.67	0.18	0.92	0.31	1.55	—	—
33	34	0.09	0.32	0.02	0.54	0.13	0.63	0.28	0.79	0.35	1.43	—	—
34	20	0.03	0.31	0.24	0.57	0.19	0.78	0.25	0.95	0.23	1.81	—	—
35	29	0.13	0.43	0.21	0.50	0.23	0.75	0.33	1.15	0.51	1.55	—	—
36	22	0.02	0.25	0.06	0.38	0.00	0.76	0.25	1.60	0.25	2.20	—	—
37	29	0.02	0.24	0.08	0.40	0.34	0.52	0.17	0.61	0.40	1.17	—	—
38	35	0.07	0.32	0.06	0.45	0.12	0.49	0.32	0.68	0.35	1.25	—	—
39	36	0.02	0.28	0.12	0.42	0.11	0.67	0.17	0.97	0.31	1.51	—	—
40	38	0.05	0.33	0.07	0.43	0.06	0.71	0.05	1.30	0.20	2.38	—	—
41	29	0.03	0.31	0.08	0.40	0.00	0.52	0.21	0.96	0.39	1.80	—	—
Mean		0.06	0.30	0.12	0.43	0.18	0.62	0.25	0.95	0.34	1.67	—	—
S.E.		±0.02	±0.01	±0.02	±0.02	±0.03	±0.03	±0.03	±0.06	±0.03	±0.09	—	—
n		18	18	18	18	18	18	18	18	18	18	—	—

Table 6. *Pulse rate (per min.) and systolic blood pressure (mm Hg) in healthy women at rest and during muscular work with successively increased loads.*

Case No.	Age	Resting		During work								5′ after work		10′ after work	
				200 kgm/min. 6′		400 kgm/min. 6′		600 kgm/min. 6′		800 kgm/min. 6′					
		Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure
1	23	60	115	125	145	170	150	–	–	–	–	77	115	68	115
2	21	91	125	140	175	165	180	–	–	–	–	111	125	103	120
3	22	64	105	120	125	155	145	–	–	–	–	75	110	–	–
4	23	95	130	124	140	165	155	–	–	–	–	103	130	96	130
Mean		78	119	127	146	164	158	–	–	–	–	92	120	89	122
S.E.		±9.0	±5.5	±4.4	±10.5	±3.1	±7.8	–	–	–	–	±9.1	±4.6	±10.7	±4.4
n		4	4	4	4	4	4	–	–	–	–	4	4	3	3
5	24	83	120	116	140	147	160	190	170	–	–	104	120	100	115
6	19	65	115	100	130	120	150	143	170	–	–	67	125	68	125
7	19	72	115	116	155	136	160	165	180	–	–	85	120	70	115
8	17	52	135	104	170	131	165	147	175	–	–	69	135	65	130
9	48	74	120	99	140	125	160	150	175	–	–	94	130	100	125
10	28	74	115	109	140	127	160	160	155	–	–	82	120	85	115
11	22	73	110	120	140	130	150	165	170	–	–	73	120	67	110
12	24	67	115	100	150	115	160	147	165	–	–	63	120	64	125
13	26	68	115	122	135	155	145	190	155	–	–	100	110	96	105
14	19	86	120	122	135	136	140	165	150	–	–	100	120	93	93
Mean		71	118	111	144	132	155	162	167	–	–	84	122	81	116
S.E.		±3.0	±3.1	±3.0	±3.7	±3.8	±2.6	±5.3	±3.2	–	–	±4.8	±2.1	±4.9	±3.5
n		10	10	10	10	10	10	10	10	–	–	10	10	10	10
15	29	65	110	87	130	110	145	138	170	167	180	86	120	81	115
16	25	85	140	115	180	118	175	137	180	165	200	86	140	87	140
17	19	64	120	103	130	122	140	143	160	175	170	83	130	–	–
18	21	75	115	116	135	130	150	170	170	185	180	95	110	92	105
Mean		72	121	105	144	120	153	147	170	173	183	88	125	87	120
S.E.		±4.9	±6.6	±6.8	±12.1	±4.2	±7.8	±7.7	±4.1	±4.5	±6.3	±2.6	±6.5	±3.2	±10.4
n		4	4	4	4	4	4	4	4	4	4	4	4	3	3

Table 7. *Plasma levels (μg/litre) of adrenaline (A) and noradrenaline (NA) in healthy women at rest and during muscular work with successively increased loads.*

S.E.		±1.5	±0.8	±6.8	±12.1	±4.2	±7.8	±7.7	±4.1	±4.5	±6.3	±2.6	±6.5	±3.2	±10.4
n		4	4	4	4	4	4	4	4	4	4	4	4	3	3

Table 7. *Plasma levels (μg/litre) of adrenaline (A) and noradrenaline (NA) in healthy women at rest and during muscular work with successively increased loads.*

Case No.	Age	Resting		During work: 200 kgm/min. 6′		400 kgm/min. 6′		600 kgm/min. 6′		800 kgm/min. 6′		5′ after work		10′ after work	
		A	NA	A	NA	A	NA	A	NA	A	NA	A	NA	A	NA
1	23	0.09	0.22	0.13	0.31	0.34	0.56	–	–	–	–	0.14	0.29		
2	21	0.10	0.48	0.16	0.62	0.19	0.93	–	–	–	–	0.14	0.58		
3	22	0.01	0.38	0.12	0.48	0.20	0.59	–	–	–	–	–	–		
4	23	0.02	0.39	0.16	0.59	0.18	0.75	–	–	–	–	–	–		
Mean		0.06	0.37	0.14	0.50	0.23	0.71	–	–	–	–	0.14	0.43		
S.E.		±0.02	±0.05	±0.01	±0.07	±0.04	±0.08	–	–	–	–	±0.00	±0.15		
n		4	4	4	4	4	4	–	–	–	–	2	2		
5	24	0.07	0.27	0.09	0.30	0.10	0.45	0.17	0.70	–	–	–	–	–	–
6	19	0.01	0.26	0.03	0.32	0.01	0.42	0.01	0.62	–	–	–	–	–	–
7	19	0.01	0.28	0.01	0.34	0.24	0.47	0.17	0.62	–	–	0.00	0.30	–	–
8	17	0.09	0.30	0.03	0.36	0.05	0.56	0.07	0.72	–	–	0.02	0.25	–	–
9	48	0.01	0.25	0.24	0.36	0.29	0.38	0.31	0.73	–	–	–	–	0.07	0.26
10	28	0.08	0.29	0.12	0.38	0.22	0.75	0.16	1.29	–	–	–	–	0.01	0.30
11	22	0.03	0.37	0.02	0.45	0.10	0.55	0.23	0.95	–	–	–	–	0.05	0.37
12	24	0.03	0.37	0.06	0.44	0.08	0.50	0.10	0.76	–	–	0.04	0.25	–	–
13	26	0.10	0.64	0.17	0.84	0.31	0.96	0.34	1.57	–	–	–	–	–	–
14	19	0.06	0.47	0.11	0.56	0.14	0.79	0.22	1.16	–	–	0.16	0.40	–	–
Mean		0.05	0.35	0.09	0.44	0.15	0.58	0.18	0.91	–	–	0.05	0.30	0.04	0.31
S.E.		±0.01	±0.04	±0.02	±0.05	±0.03	±0.06	±0.03	±0.10	–	–	±0.04	±0.04	±0.02	±0.03
n		10	10	10	10	10	10	10	10	–	–	4	4	3	3
15	29	0.12	0.27	0.11	0.38	0.32	0.42	0.26	0.87	0.28	1.20	–	–	–	–
16	25	0.06	0.31	0.02	0.42	0.19	0.45	0.22	0.70	0.34	1.24	–	–	–	–
17	19	0.05	0.35	0.12	0.49	0.12	0.69	0.16	0.99	0.23	1.33	–	–	–	–
18	21	0.07	0.37	0.07	0.58	0.13	0.77	0.15	0.98	0.36	1.41	–	–	–	–
Mean		0.07	0.32	0.08	0.47	0.19	0.59	0.20	0.88	0.30	1.30	–	–	–	–
S.E.		±0.01	±0.02	±0.02	±0.04	±0.05	±0.09	±0.03	±0.07	±0.03	±0.05	–	–	–	–
n		4	4	4	4	4	4	4	4	4	4	—	—	—	—

Table 8. *Plasma levels (μg/litre) of adrenaline (A) and rate (per min.) and blood pressure (mm Hg) ch*

I. Healthy subjects (tilting at 90° for 10 minutes

Case No.	Age	Sex	Recumbency					After 2 minutes			
			Pulse	Blood pressure		A	NA	Pulse	Blood pressure		A
				syst.	diast.				syst.	diast.	
1	29	F	72	110	60	0.03	0.17	92	120	70	0.04
2	24	F	84	115	70	–0.01	0.35	100	115	80	0.05
3	33	F	96	120	75	0.10	0.23	112	115	100	0.10
4	33	F	76	115	65	0.01	0.32	92	115	85	0.01
5	65	F	76	150	80	–0.01	0.37	96	150	90	0.02
6	40	F	88	120	75	0.11	0.26	88	115	90	0.11
7	38	F	76	115	70	0.09	0.31	94	160	75	0.08
8	41	F	90	105	65	0.11	0.34	92	105	75	0.19
9	40	M	88	150	90	–0.04	0.38	112	160	100	–0.04
10	33	F	60	115	70	0.14	0.51	78	95	80	0.14
11	46	F	84	120	85	0.01	0.36	106	120	90	–
12	26	F	64	105	65	–0.05	0.36	78	105	70	–
13	28	M	60	120	60	0.08	0.42	78	115	80	–
14	53	M	72	125	85	0.09	0.42	88	125	85	–
15	19	M	68	115	65	0.06	0.43	80	115	80	–
16	38	M	88	110	70	0.04	0.50	104	115	100	–
17	28	M	68	120	65	–0.02	0.41	100	120	80	–
Mean			77	119	71	0.04	0.36	94	121	84	0.07
S.E.			±2.7	±3.1	±2.2	±0.01	±0.02	±2.7	±4.4	±2.4	±0.02
n			17	17	17	17	17	17	17	17	10

(A) and noradrenaline (NA) with pulse Hg) changes of individual subjects tilted.

minutes).

Tilting period										
	After 5 minutes					After 10 minutes				
NA	Pulse	Blood pressure		A	NA	Pulse	Blood pressure		A	NA
		syst.	diast.				syst.	diast.		
0.18	84	115	70	–	–	94	115	65	0.06	0.34
0.57	96	100	80	–	–	108	105	75	0.03	0.67
0.24	114	115	100	–	–	116	110	95	0.05	0.55
0.29	92	110	80	–	–	92	110	85	0.07	0.58
0.41	96	150	90	–	–	96	135	90	0.08	0.59
0.26	84	115	80	–	–	90	115	80	0.04	0.49
0.29	100	120	100	–	–	96	125	100	0.11	0.58
0.28	96	105	75	–	–	108	105	75	0.25	0.49
0.39	116	160	80	–	–	108	140	90	0.01	0.43
0.71	72	95	80	–	–	72	95	75	0.24	0.78
–	106	120	90	0.03	0.46	104	110	85	0.13	0.54
–	88	105	65	–0.06	0.64	90	105	70	–0.06	0.61
–	84	120	80	0.08	0.63	86	120	85	0.10	0.68
–	92	130	100	0.11	0.48	96	125	95	0.22	0.74
–	82	115	80	0.13	0.73	96	115	80	0.20	0.92
–	104	120	95	0.11	0.56	102	120	90	0.26	0.72
–	90	120	85	0.01	0.67	92	120	80	0.07	0.82
0.36	94	119	84	0.06	0.60	97	116	83	0.11	0.62
±0.05	±2.8	±3.9	±2.5	±0.03	±0.04	±2.5	±2.8	±2.3	±0.02	±0.03
10	17	17	17	7	7	17	17	17	17	17

II. a) Subjects with orthostatic hypo-

Case No.	Age	Sex	Recumbency					Tilting period After 2 minutes			
			Pulse	Blood pressure syst.	Blood pressure diast.	A	NA	Pulse	Blood pressure syst.	Blood pressure diast.	A
18	33	F	70	100	60	0.03	0.29	100	95	75	–0.03
19	22	F	66	105	60	0.03	0.37	90	105	80	0.03
20	27	M	60	140	70	–0.01	0.43	92	135	85	0.21
21	35	F	72	125	80	–0.12	0.51	96	125	80	0.06
22	47	F	56	120	70	0.16	0.38	64	110	80	–
23	17	F	84	115	55	0.09	0.31	112	105	55	–
24	20	F	84	110	70	0.11	0.37	116	110	90	–
25	54	F	76	120	75	0.06	0.44	80	105	75	–
26	22	F	92	115	60	0.05	0.43	120	110	65	–
27	39	F	94	140	100	0.05	0.39	96	150	90	–
28	57	M	92	125	65	0.07	0.36	112	120	70	–
29	27	M	64	110	60	0.04	0.28	87	100	80	–
30	40	M	68	105	75	0.11	0.36	108	105	80	–
31	42	M	96	125	90	0.02	0.57	140	110	95	–
Mean			77	118	71	0.05	0.39	101	113	79	0.07
S.E.			±3.6	±3.3	±3.4	±0.02	±0.02	±5.1	±3.9	±2.8	±0.05
n			14	14	14	14	14	14	14	14	4

II. b) Subjects with orthostatic hypo-

Case No.	Age	Sex	Recumbency					Tilting After 2 minutes		
			Pulse	Blood pressure syst.	Blood pressure diast.	A	NA	Pulse	Blood pressure syst.	Blood pressure diast.
32	22	M	70	110	60	0.03	0.31	104	102	55
33	18	M	94	125	50	0.11	0.36	140	120	60
34	27	M	66	110	75	0.03	0.53	80	115	55
35	20	F	84	105	65	0.04	0.30	116	90	75
36	34	F	72	100	70	0.02	0.23	107	80	75
37	27	F	64	115	70	0.04	0.30	102	110	80
38	21	M	64	135	70	0.05	0.53	80	125	90
Mean			73	114	66	0.05	0.37	104	107	70
S.E.			±4.3	±4.6	±3.2	±0.01	±0.04	±7.9	±6.1	±5.1
n			7	7	7	7	7	7	7	7

tension (tilting at 90° for 10 minutes).

Tilting period										
	After 5 minutes					After 10 minutes				
NA	Pulse	Blood pressure		A	NA	Pulse	Blood pressure		A	NA
		syst.	diast.				syst.	diast.		
0.51	102	95	75	–	–	112	90	80	0.09	0.70
0.35	92	90	65	–	–	112	65	60	0.05	0.69
0.28	104	140	100	–	–	108	140	100	0.21	0.60
0.72	108	120	90	–	–	104	115	95	0.03	0.90
–	72	120	80	0.06	0.54	88	110	80	0.06	0.72
–	124	100	60	0.13	0.54	140	85	70	0.11	0.84
–	102	110	90	0.08	0.74	132	95	90	0.02	1.18
–	96	100	80	0.07	0.56	100	90	80	0.06	0.67
–	126	110	65	0.16	0.94	130	105	65	0.22	1.10
–	124	125	100	0.10	0.66	128	125	90	0.08	0.84
–	112	115	70	0.09	0.59	124	110	70	0.12	0.71
–	92	110	85	–0.01	0.36	108	100	80	0.04	0.52
–	108	105	80	0.11	0.46	104	105	90	0.09	0.62
–	148	110	95	0.09	1.02	160	105	95	0.11	1.33
0.46	108	111	81	0.09	0.64	118	103	82	0.09	0.82
±0.10	±5.0	±3.5	±3.5	±0.01	±0.07	±5.0	±4.9	±3.3	±0.02	±0.06
4	14	14	14	10	10	14	14	14	14	14

tension (tilting at 90° for 5 minutes).

period					Recumbency				
After 5 minutes					5 minutes after tilting				
Pulse	Blood pressure		A	NA	Pulse	Blood pressure		A	NA
	syst.	diast.				syst.	diast.		
108	unmeasurable		0.11	0.74	–	–	–	0.01	0.36
132	"		0.08	0.90	–	–	–	0.02	0.42
84	"		0.12	0.93	–	–	–	–	–
120	80	65	0.03	0.65	–	–	–	0.12	0.61
75	95	80	0.06	0.72	–	–	–	–	–
120	unmeasurable		0.11	0.77	–	–	–	0.14	0.43
88	125	85	0.25	1.12	92	120	80	0.06	0.69
104	–	–	0.11	0.83	92	120	80	0.07	0.50
±8.2	–	–	±0.03	±0.06	–	–	–	±0.03	±0.06
7			7	7	1	1	1	5	5

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